THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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VOLUME LXXI
AUGUST TO DECEMBER, 1936

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN is issued six times a year. Single numbers, $1.75. Subscription per volume (3 numbers), $4.50.

Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa. Agent for Great Britain: Wheldon & Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W.C. 2.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Mass., between June 1 and October 1 and to the Institute of Biology, Divinity Avenue, Cambridge, Mass., during the remainder of the year.

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

LANCASTER PRESS, INC., LANCASTER, PA.

Science
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INSTRUCTIONS TO AUTHORS

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Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Vol. LXXI, No. 1 August, 1936

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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FORTY-EIGHTH YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio*, who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members is fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1935.

The accounts have been audited by Messrs. Seaman, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1935, the book value of the Endowment Funds in the hands of the Central Hanover Bank and Trust Company as Trustee, was

General Fund,	Securities	$ 884,683.36
	Cash	26,482.57
Library Fund,	Securities	193,582.06
	Cash	101.19
Total book value		$1,104,849.18

The income collected from these Funds was as follows:

General Endowment	$40,479.31
Library Fund	7,471.45
	$47,950.76

an increase of a little more than one thousand dollars ($1,000) over the income from these Funds in 1934.

The income in arrears, some of which may never be collected, was on December 31, 1935:

General Fund	$13,944.05
Library Fund	4,150.00

The extra dividends from the stock of the General Biological Supply

House have continued; the total dividends received from that Company amounted during the year to $10,922.00.

Retirement Fund. A total of $4,560 in pensions and benefits was paid out of the Retirement Fund. The Fund at the end of the year consisted of securities of the book value of $21,129.42
Cash 48.37

Total $21,177.79
Income in arrears on December 31 was $321.98

The land, buildings, equipment and library (exclusive of the Gansett and Devil's Lane tracts) represented an investment of ... $1,727,703.99
less reserve for depreciation of 445,913.96

$1,281,790.03

Expenses including $43,625.42 reserve for depreciation exceeded income by $14,794.20. There was expended from current funds $22,087.34 for plant account, mostly for equipment and books.

The property of the Laboratory is free and clear of mortgages. At the end of the year it owed on open account $4,427.07 and had accounts receivable of $7,345.83 and $13,541.05 in cash and bank accounts.

Following is the balance sheet as of December 31, 1935, the condensed statement of income and outgo, and the surplus account, all as set out by the accountants.

EXHIBIT A

Marine Biological Laboratory Balance Sheet, December 31, 1935

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee—Schedules I-a and I-b		$1,104,849.18	
Securities and Cash—Minor Funds—Schedule II		8,108.24	$1,112.957.42
Plants Assets:			
Land—Schedule IV	$ 98,103.05		
Buildings—Schedule IV	1,226,910.53		
Equipment—Schedule IV	167,022.12		
Library—Schedule IV	235,668.29	$1,727,703.99	
Less Reserve for Depreciation		445,913.96	
		$1,281,790.03	
Cash in Dormitory Building Fund		358.24	
Cash in Reserve Fund		24.65	$1,282,172.92

Current Assets:			
Cash		$ 13,541.05	
Accounts and Notes-Receivable		7,345.83	
Inventories			
Supply Department	$ 38,399.27		
Biological Bulletin	11,384.66	49,783.93	
Investments:			
Devil's Lane Property	$ 43,341.07		
Gansett Property	5,511.29		
Stock in General Biological Supply House, Inc.	12,700.00		
Securities and Cash—Retirement Fund—Schedule V	21,177.79	82,730.15	
Prepaid Insurance		3,126.19	
Items in Suspense (Net)		146.30	$ 156,673.45
			$2,551,803.79
Liabilities			
Endowment Funds:			
Endowment Funds—Schedule III		$1,104,849.18	
Minor Funds—Schedule III		8,108.24	$1,112,957.42
Plant Funds:			
Donations and Gifts—Schedule III		$1,029,572.61	
Other Investments in Plant from Gifts and Current Funds		252,600.31	$1,282,172.92
Current Liabilities and Surplus:			
Accounts—Payable		$ 3,763.15	
Woods Hole Oceanographic Institution		663.92	
		$ 4,427.07	
Current Surplus—Exhibit C		152,246.38	$ 156,673.45
			$2,551,803.79

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1935

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 40,479.31		$ 40,479.31
Library Fund		7,471.45		7,471.45
Gifts		400.00		400.00
Instruction	$ 7,931.82	9,770.00		1,838.18
Research	4,145.52	12,470.00		8,324.48
Evening Lectures	83.99		$ 83.99	
Biological Bulletin and Membership Dues	7,790.04	8,805.35		1,015.31

Supply Department—Schedule VI	39,772.62	42,813.25		3,040.63
Mess—Schedule VII	21,611.81	21,349.57	262.24	
Dormitories—Schedule VIII	32,424.48	12,204.18	20,220.30	
(Interest and Depreciation charged to above three Departments—See Schedules VI, VII, and VIII)	35,260.33			35,260.33
Dividends, General Biological Supply House, Inc.		10,922.00		10,922.00
Rents:				
Danchakoff Cottages	352.63	750.00		397.37
Newman Cottage	88.80	250.00		161.20
Janitor's House	65.26	225.00		159.74
Railway and Garage		52.00		52.00
Sale of Duplicate Library Sets		58.05		58.05
Sundries		14.77		14.77
Maintenance of Plant:				
Buildings and Grounds	21,333.77		21,333.77	
Chemical and Special Apparatus	13,091.90		13,091.90	
Library Department Expense	7,598.02		7,598.02	
Truck Expense	849.94		849.94	
Sundry Expense	25.00		25.00	
Workmen's Compensation Insurance	553.16		553.16	
General Expenses:				
Administration Expenses	15,208.57		15,208.57	
Endowment Fund Trustee	968.50		968.50	
Bad Debts	568.21		568.21	
Reserve for Depreciation	43,625.42		43,625.42	
	$182,829.13	$168,034.93	$124,389.02	$109,594.82
Excess of Expenses over Income carried to Current Surplus—Exhibit C		14,794.20		14,794.20
		$182,829.13		$124,389.02

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT, YEAR ENDED DECEMBER 31, 1935

Balance, January 1, 1935	$148,510.68
Add:	
Reserve for Depreciation charged to Plant Funds	43,625.42
	$192,136.10

Deduct:			
Payments from Current Funds during Year for Plant Assets as Shown in Schedule IV,			
Buildings		$ 2,012.64	
Equipment		5,229.08	
Library, Books, etc.		14,845.62	
		$22,087.34	
Less amount received for Plant Assets disposed of and charged to Current Account		690.00	
		$21,397.34	
Pensions and Allowances Paid	$4,560.00		
Less Income of Retirement Fund Received	861.82	3,698.18	
Excess of Expenses over Income for Year as shown in Exhibit B		14,794.20	39,889.72
Balance, December 31, 1935—Exhibit A			$152,246.38

Respectfully submitted,
LAWRASON RIGGS, JR.,
Treasurer

V. THE REPORT OF THE LIBRARIAN

We report for the year 1935 an increase in the Library budget of $500 specifically for the purchase of back sets, an activity practically at a standstill since 1933, when there was a cut of $5,000 from our budget. In the years 1933–34, the cost of serials being then at its maximum, the small amount that we had for back sets was used principally to retain all of our current serial titles intact. This year the expenditures were more normal in current serials and back sets and at the same time the binding was restored to normal. Library expenditures gave practically a balanced budget in each assignment apportioned as in former years: books, $300; serials, $6,000; binding, $1,500; express, $300; supplies, $500; back sets, $2,350; salaries, $7,150; total, $18,100; and applied as follows: books, $298.64; serials, $5,473.24; binding, $1,292.26; express, $97.96; supplies, $345.35; back sets, $3,496.08; salaries, $7,150.00; total, $18,153.53.

The scientific literature acquired by the Library in 1935 was therefore in great contrast to that of 1933–34, not only in back sets, 24 completed and 17 partially completed, as against 8 completed and 16 partially completed, but also in current serial titles which were increased by 74, total now received, 1,271: 359 purchased by the Marine Biological Laboratory (9 new), 37 by the Woods Hole Oceanographic Institution,

602 by exchange with the "Biological Bulletin" (21 new), 34 by exchange for the publications of the Oceanographic Institution (15 new), 225 as gifts to the former (25 new) and 14 to the latter (4 new). The Library acquired 158 new books, 93 by purchase and 41 by gift to the Marine Biological Laboratory, 21 purchased by the Woods Hole Oceanographic Institution and 3 gifts. Thirteen authors presented books and the other gifts were acquired through the courtesy of publishers.

The Library now contains 40,180 volumes and 91,641 reprints. Of the 4,478 reprints received this year, 927 were issued in serials of the year 1935. This figure is interesting because it is higher than at any time since we began in 1932 to make a separate check of the current year reprint receipts.

A new plan for current reprints will be begun this summer. They will be catalogued each day and placed for inspection on a special table in the reading room before they are filed away in the authors' boxes. We hope thus to make the new reprints more useful and at the same time to encourage our investigators to keep their files of reprints in the Library to date and complete.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-eighth session of the Marine Biological Laboratory for the year 1935.

1. *Attendance.* Following the sharp decline in attendance in 1932 from the previous high figure of 362, the number of regularly registered investigators at the Laboratory has remained almost stationary, with only minor fluctuations from year to year. The exact figures for the past 5 years are: 362 in 1931, 314 in 1932, 319 in 1933, 323 in 1934 and 315 in 1935. The number of students in the courses has likewise remained practically constant at the limit set by the sizes of the available class-rooms. The total number of students and investigators together, after allowing for duplications, of 429 in 1935 represents approximately the optimum for the present facilities of the Laboratory, since it is just sufficient to fill all the available space comfortably and without undue crowding. Particularly noteworthy in 1935 was the large number of institutions represented by investigators. Both this number, which was 111, and the total of 143 for investigators and students combined, were the largest in the history of the Laboratory. The usual tabulation of the seasonal distribution of attendance at ten-day intervals for the past 9 years follows:

		1927	1928	1929	1930	1931	1932	1933	1934	1935
May	30	7	15	9	6	6	8	11	12	11
June	10	50	64	55	50	51	54	46	54	43
"	20	114	140	139	153	153	127	129	137	127
"	30	212	240	197	208	217	172	184	196	174
July	10	247	281	238	253	258	225	235	249	226
"	20	247	282	242	250	273	245	253	256	232
"	30	245	272	249	253	281	248	255	248	257
August	10	234	250	256	254	302	257	261	264	245
"	20	208	226	243	245	280	236	244	250	235
"	30	168	183	220	204	239	190	205	211	192
September	10	110	112	157	122	136	129	117	93	94
"	20	50	43	59	44	69	58	45	38	26
"	30	12	14	14	8	14	13	12	9	11

2. *The Report of the Treasurer.* In 1935, for the first time in four years, the annual decline in the income of the Laboratory from its endowment funds has been replaced by a slight increase. Though this increase amounted to slightly less than a thousand dollars, and though the total income from endowment of $47,950.76 is still nearly ten thousand dollars below that of pre-depression years, it is nevertheless very gratifying to be able to report an actual reversal of the previous trend. An inspection of Exhibit B from the Auditors' Report also reveals an increase of approximately $1,000.00 in the net income from the Supply Department (though the gross receipts were less than those for 1934), and similarly, for the first time in 4 years, there has been a slight but encouraging increase in the receipts from the rental of research space. Particularly helpful to the Laboratory at a time when its regular income was at nearly its lowest level since the establishment of its present endowment fund was a second special dividend from the General Biological Supply House, this dividend representing profits accumulated but not distributed during a period when business uncertainties made a relatively large reserve seem desirable.

Though in view of all the circumstances the present financial situation of the Laboratory is very satisfactory, it should nevertheless be noted that the annual gross income in 1935 was nearly forty thousand dollars less than that in 1931 and that throughout the period of the depression the budget has been kept balanced only by drastic economies of various sorts, some of which cannot much longer be continued without detriment to the scientific activities of the institution. It should likewise be remembered that for a considerable number of years to come the income of the Laboratory will be adversely affected by the gradual maturing of securities in its endowment fund, the proceeds from which must of necessity be reinvested at lower rates of interest. While the financial

position of the Laboratory is therefore sound, and indeed extremely fortunate as compared with that of most other scientific and educational institutions, it is not at present such as to justify any departure from the very conservative policy with regard to expenditures that has been followed for the past four years.

3. *The Report of the Librarian.* Though it has not yet become possible to restore the earlier rate of growth of the library, temporarily checked by the reduction in the income from endowment funds, it is nevertheless encouraging to record very satisfactory gains during the past year. In particular, the increase in the number of journals currently received, amounting to 74 for the year in question, has been the largest for any single year since 1931. Substantial progress in the completion of back sets of journals has also been made. As the number of reprints in the library approaches the 100,000 mark, the attention of all members of the Corporation is invited to the desirability of transferring to this collection reprints in their own possession which are little or not at all used, but which might be of great value to other workers at the Laboratory. The growth of the Library since 1925 is concisely summarized in the following table:

	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935
Serials received currently	500	628	764	874	985	1,060	1,080	1,126	1,137	1,197	1,271
Total number of bound volumes	15,000	18,200	22,800	26,500	28,300	31,500	33,800	36,000	37,400	38,600	40,200
Reprints	25,000	38,000	43,000	51,000	59,000	64,000	70,000	76,000	81,000	86,000	92,000

4. *Lectures and Scientific Meetings.* A complete list of the various lectures and scientific papers presented during the summer of 1935 will be found below (pages 28 to 32). The number of general lectures was 11, while there were also held 8 evening meetings and an all-day scientific session devoted to work accomplished at the Laboratory during 1935, at which 67 shorter papers were presented and discussed. Abstracts of most of these shorter papers, which cover a wide range of scientific activities, will be found in the Biological Bulletin for October, 1935.

5. *Apparatus Committee.* The great diversity in the character of the investigations carried on at the Marine Biological Laboratory and particularly the increasing complexity of the apparatus needed for their successful prosecution has for some time rendered desirable an advisory body, analogous to the Library Committee, the members of which can give advice concerning the purchase of special types of equipment of whose use they have expert knowledge, and who in cooperation with the Technical Manager can determine questions of general policy with regard to the most effective use of the valuable apparatus already in the

possession of the Laboratory. To meet this need, following authorization by the Executive Committee, President Lillie in the early summer of 1935 appointed a Committee of three consisting of Drs. Garrey, Harvey and Heilbrunn, which was later enlarged to the following membership: Drs. W. R. Amberson, D. J. Edwards, W. E. Garrey, E. N. Harvey, M. H. Jacobs and L. V. Heilbrunn, Chairman. During the summer of 1935 extremely useful work was accomplished by the original committee of three in making a general survey of all the apparatus belonging to the Laboratory and in securing from individual investigators a large number of valuable suggestions. At a meeting of the full committee held in September a series of general recommendations based on the information so obtained was drawn up. These recommendations should serve as a very sound basis for the future policies of the Laboratory in this important and highly technical field.

6. *Board of Trustees.* At the meeting of the Corporation held on Tuesday, August 13, 1935 the long and valuable services on the Board of Trustees of Professor G. H. Parker, whose membership began in 1908, and of Professor W. M. Wheeler, whose membership began in 1919, received recognition by the election of both to the permanent position of Trustee Emeritus. To fill the vacancies thus created the Corporation elected Professor C. E. McClung (Class of 1936) and Dr. A. H. Sturtevant (Class of 1939) respectively. Professor Laurence Irving was also chosen to succeed Professor W. C. Curtis as a member of the Class of 1939.

There are appended as parts of the report:

1. The Staff, 1935.
2. Investigators and Students, 1935.
3. A Tabular View of Attendance, 1931–1935.
4. Subscribing and Cooperating Institutions, 1935.
5. Evening Lectures, 1935.
6. Shorter Scientific Papers, 1935.
7. Members of the Corporation, August, 1935.

Respectfully submitted,

M. H. JACOBS,
Director.

1. THE STAFF, 1935

MERKEL H. JACOBS, *Director*, Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology Emeritus, The University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy Emeritus, Harvard University.
E. B. WILSON, Professor of Zoölogy, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

T. H. BISSONNETTE, Professor of Biology, Trinity College.
E. C. COLE, Professor of Biology, Williams College.
C. E. HADLEY, Associate Professor of Biology, New Jersey State Teachers College at Montclair.
F. R. KILLE, Instructor in Zoölogy, Swarthmore College.
S. A. MATTHEWS, Associate in Anatomy, School of Medicine, University of Pennsylvania.
O. E. NELSEN, Instructor in Zoölogy, University of Pennsylvania.
L. P. SAYLES, Assistant Professor of Biology, College of the City of New York.

JUNIOR INSTRUCTORS

A. J. WATERMAN, Assistant Professor of Biology, Williams College.
F. H. WOODS, Assistant Professor of Zoölogy, University of Missouri.

PROTOZOÖLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
ELIZABETH DRUMTRA, Instructor in Zoölogy, Wilson College.
G. W. KIDDER, Instructor in Zoölogy, College of the City of New York.

EMBRYOLOGY

I. Investigation

(*See Zoölogy*)

II. Instruction

L. G. Barth, Instructor of Experimental Zoölogy, Columbia University.
Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Benjamin H. Grave, Professor of Biology, De Pauw University.
Leigh Hoadley, Professor of Zoölogy, Harvard University. (Absent in 1935.)
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.
Oscar Schotté, Assistant Professor of Biology, Amherst College.

PHYSIOLOGY

I. Investigation

William R. Amberson, Professor of Physiology, University of Tennessee.
Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, The University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

Robert Chambers, Professor of Biology, New York University.
J. K. W. Ferguson, Assistant Professor of Physiology, University of Western Ontario.
Rudolf Höber, Visiting Professor of Physiology, University of Pennsylvania.
Laurence Irving, Professor of Experimental Biology, University of Toronto.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.
C. Ladd Prosser, Assistant Professor of Physiology, Clark University.

Junior Instructors

Kenneth Fisher, Demonstrator in Biology, University of Toronto.
F. J. M. Sichel, Instructor in Zoölogy, University of Pennsylvania.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Michigan.
G. W. Prescott, Assistant Professor of Biology, Albion College.

GENERAL OFFICE

F. M. MacNaught, Business Manager.
Polly L. Crowell, Assistant.
Edith Billings, Secretary.

RESEARCH SERVICE AND GENERAL MAINTENANCE

Samuel E. Pond, Technical Manager.
Oscar W. Richards, Chemical Service.
G. Failla, X-Ray Physicist.
Thomas E. Larkin, Superintendent.
William Hemenway, Carpenter.
Lester F. Boss, Research Technician.
J. D. Graham, Glassblower.
P. H. Liljestrand, Assistant.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Doris Endrejat, Mary A. Rohan, Assistants.

SUPPLY DEPARTMENT

James McInnis, Manager.
Milton B. Gray, Collector.
A. M. Hilton, Collector.
A. W. Leathers, Shipping Department.
Geoffrey Lehy, Collector.
Walter Kahler, Collector.
Ruth S. Crowell, Secretary.
Anna N. Hall, Secretary.

MUSEUM

George M. Gray, Curator Emeritus.

2. INVESTIGATORS AND STUDENTS, 1935

Independent Investigators

Abramowitz, Alexander A., Assistant in Biology, Harvard University.
Adams, James A., Fellow, Iowa State College.
Adolph, E. F., Associate Professor of Physiology, University of Rochester, School of Medicine.
Albaum, Harry G., Graduate Student, Columbia University.
Alexander, Gordon, Associate Professor of Biology, University of Colorado.
Alvey, Clifford H., Instructor in Parasitology, Purdue University.
Amberson, William R., Professor of Physiology, College of Medicine, University of Tennessee.
Anderson, R. L., Professor of Biology, Johnson C. Smith University.
Appel, F. W., Associate Professor of Biology, St. John's College.
Baker, Clinton L., Professor of Biology, Southwestern.

BALL, ERIC G., Associate, Department of Physiological Chemistry, Johns Hopkins Medical School.
BARTH, L. G., Instructor in Embryology, Columbia University.
BISSONNETTE, T. HUME, Professor and Head of Biology Department, Trinity College.
BOSTIAN, C. H., Assistant Professor of Zoölogy, North Carolina State College.
BOWEN, R. E., Assistant Professor of Biology, Long Island University.
BOYD, JAMES D., Rockefeller Fellow, Queens University, Belfast, Ireland.
BOZLER, EMIL, Fellow in Medical Physics, Johnson Foundation, University of Pennsylvania.
BRADLEY, H. C., Professor of Physiological Chemistry, University of Wisconsin.
BRINLEY, FLOYD J., Assistant Professor of Zoölogy, North Dakota State College.
BROWN, DUGALD E. S., Assistant Professor of Physiology, New York University.
BUDINGTON, R. A., Professor of Zoölogy, Oberlin College.
BURTON, A. C., General Education Training Fellowship, University of Pennsylvania.
CABLE, RAYMOND M., Associate Professor of Biology, Berea College.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CARLSON, J. GORDON, Instructor in Biology, Bryn Mawr College.
CARPENTER, ESTHER, Instructor in Zoölogy, Smith College.
CARPENTER, RUSSELL L., Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University.
CATTELL, WARE, Associate Editor, Scientific Monthly.
CHAMBERS, ROBERT, Research Professor of Biology, Washington Square College, New York University.
CHENEY, RALPH H., Professor of Biology, Long Island University.
CLARK, ELEANOR LINTON, University of Pennsylvania, Medical School.
CLARK, ELIOT R., Professor of Anatomy, University of Pennsylvania, Medical School.
CLARK, LEONARD B., Assistant Professor of Biology, Union College.
CLOWES, G. H. A., Director of Research, Lilly Research Laboratories.
COE, W. R., Professor of Biology, Yale University.
COLE, ELBERT C., Professor of Biology, and Chairman of Department, Williams College.
CONKLIN, EDWIN G., Professor Emeritus of Biology, Princeton University.
COONFIELD, B. R., Assistant Professor, Brooklyn College.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COSTELLO, DONALD P., National Research Fellow in Zoölogy, Hopkins Marine Station.
COTUI, FRANK W., Associate Professor, Research Surgery, New York University, College of Medicine.
COWLES, R. P., Professor of Zoölogy, Johns Hopkins University.
CROASDALE, HANNAH T., Graduate Student, University of Pennsylvania.
CROWELL, PRINCE S., JR., Instructor, Harvard University.
DANFORTH, LOUISE, Graduate Alumna, American University.
DAVIS, J. E., Research Assistant, The University of Chicago.
DIETER, CLARENCE D., Acting Head of Biology, Washington and Jefferson College.
DILLER, WILLIAM F., Instructor in Zoölogy, Dartmouth College.
DONALDSON, HENRY H., Member, Wistar Institute.
DOYLE, WILLIAM L., General Education Board Fellow, The Johns Hopkins University.
DREYER, W. A., Instructor in Zoölogy, University of Cincinnati.
DRUMTRA, ELIZABETH, Assistant in Zoölogy, Barnard College, Columbia.
DUBOIS, EUGENE F., Professor of Medicine, Cornell University Medical College.
DUBUY, HERMAN G., Fellow, Harvard Medical School.

EDWARDS, DAYTON J., Associate Professor of Physiology, Cornell University Medical College.
FENN, WALLACE O., Professor of Physiology, University of Rochester, School of Medicine and Dentistry.
FERGUSON, J. K. W., Lecturer in Physiology, University of Western Ontario Medical School.
FISCHER, ERNST, Visiting Associate in Physiology, Medical School, University of Rochester.
FLEISHER, MOYER S., Professor of Bacteriology and Hygiene, St. Louis University.
FRY, HENRY J., Visiting Investigator, Cornell University Medical College.
FUCHS, WILLIAM B., American University.
GARREY, W. E., Professor of Physiology, Medical School of Vanderbilt University.
GOJDICS, MARY, Assistant Professor of Biology, Duchesne College.
GOODRICH, H. B., Professor of Biology, Wesleyan University.
GOTTSCHALL, GERTRUDE G., Assistant in Biochemistry, Cornell University Medical College.
GRAVE, B. H., Director, Department of Zoölogy, DePauw University.
GRAVE, CASWELL, Professor of Zoölogy, Washington University.
GUEST, GEORGE M., Associate Professor of Pediatrics, University of Cincinnati Medical College.
HADLEY, CHARLES E., Associate Professor of Biology, New Jersey State Teachers' College at Montclair.
HAGUE, FLORENCE, Assistant Professor of Zoölogy, Sweet Briar College.
HANSTRÖM, BERTIL, Professor of Zoölogy, Director of the Zoological Institute. University of Lund, Sweden.
HARKINS, HENRY H., Research Chemist, U. S. Rubber Company.
HARNLEY, MORRIS H., Assistant Professor, Washington Square College, New York University.
HARVEY, ETHEL BROWNE, Princeton University.
HASLER, ARTHUR D., Graduate Assistant, University of Wisconsin.
HAYWOOD, CHARLOTTE, Associate Professor of Physiology, Mount Holyoke College.
HEILBRUNN, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
HENDEE, ESTHER C., Research Associate, University of California.
HENSHAW, PAUL S., Biophysicist, Memorial Hospital.
HESS, WALTER N., Professor of Biology and Head of Department, Hamilton College.
HIBBARD, HOPE, Assistant Professor of Zoölogy, Oberlin College.
HILL, SAMUEL E., Assistant in General Physiology, Rockefeller Institute.
HÖBER, RUDOLF, Visiting Professor of Physiology, University of Pennsylvania.
HODGE, CHARLES, 4th, Instructor, Temple University.
HOLTER, HEINZ, Research Worker, Carlsberg Laboratory, Copenhagen.
HOOK, SABRA J., Assistant Professor of Zoölogy, University of Rochester.
HOWE, H. E., Editor, Industrial and Engineering Chemistry.
HUGGINS, JOHN R., Graduate Student, University of Pennsylvania.
HUNT, REID, Professor of Pharmacology, Harvard Medical School.
HURSH, JOHN B., Fellow, Rochester Medical School.
HYMAN, LIBBIE H., American Museum of Natural History.
IVES, PHILIP T., California Institute of Technology.
IRVING, LAURENCE, Professor of Experimental Biology, University of Toronto.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JENKINS, GEORGE B., Professor of Anatomy, George Washington University.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt University School of Medicine.
JONES, E. ELIZABETH, Instructor in Zoölogy, Wellesley College.

JONES, RUTH MCCLUNG, Instructor in Zoölogy and Botany, Swarthmore College.
KAUFMANN, BERWIND P., Professor of Botany, University of Alabama.
KEIL, ELSA M., Assistant Professor of Zoölogy, New Jersey College for Women.
KIDDER, GEORGE W., Instructor, College of the City of New York.
KILLE, FRANK R., Instructor in Zoölogy, Swarthmore College.
KILPATRICK, MARTIN, Assistant Professor of Chemistry, University of Pennsylvania.
KILPATRICK, MARY L., University of Pennsylvania.
KINDRED, JAMES E., Associate Professor of Histology and Embryology, University of Virginia Medical School.
KLEINHOLZ, L. H., Austin Fellow, Harvard University.
KNOWER, HENRY MCE., Research Associate in Biology, Yale University.
KNOWLTON, FRANK P., Professor of Physiology, College of Medicine, Syracuse University.
KREEZER, GEORGE, Research Associate, Training School at Vineland, New Jersey.
KUYPER, ADRIAN C., Instructor, State University of Iowa.
LANCEFIELD, DONALD E., Associate Professor of Zoölogy, Columbia University.
LANCEFIELD, REBECCA C., Associate in Bacteriology, Rockefeller Institute.
LAUG, EDWIN P., Assistant in Physiology, University of Pennsylvania.
LILLIE, PROF. FRANK R., Professor of Embryology, Emeritus, The University of Chicago.
LILLIE, PROF. RALPH S., Professor of General Physiology, The University of Chicago.
LONG, MARGARET E., Graduate Student, University of Pennsylvania.
LUCAS, ALFRED M., Assistant Professor of Zoölogy, University of Iowa.
LUCAS, MIRIAM SCOTT, Instructor in Cytology, Washington University School of Medicine.
MAGRUDER, SAMUEL R., Laboratory Instructor, University of Cincinnati.
MANWELL, REGINALD D., Associate Professor of Zoölogy, Syracuse University.
MARSLAND, DOUGLAS A., Assistant Professor of Biology, Washington Square College, New York University.
MARTIN, EARL A., Chairman of Biology Department, Brooklyn College.
MARQUETTE, WILLIAM G., Graduate Student, Columbia University.
MAST, S. O., Professor of Zoölogy, The Johns Hopkins University.
MATHEWS, ALBERT P., Professor of Biochemistry, University of Cincinnati.
MATTHEWS, SAMUEL A., Associate Professor of Anatomy, University of Pennsylvania.
MCCLUNG, C. E., Director, Department of Zoölogy, University of Pennsylvania.
MICHAELIS, LEONOR, Member, Rockefeller Institute for Medical Research.
MONNÉ, LUDWIK, Rockefeller Fellow, University of Lwow, Poland.
MORGAN, LILIAN V., Pasadena, California.
MORGAN, T. H., Professor of Experimental Zoölogy, California Institute of Technology.
MORILL, CHARLES V., Associate Professor of Anatomy, Cornell University Medical College.
MURATORI, GIULIO, Rockefeller Fellow, Institute of Anatomy, R. University of Padova, Italy.
NAVEZ, ALBERT E., Lecturer in Physiology, Harvard University.
NELSEN, OLIN E., Assistant Professor of Zoölogy, University of Pennsylvania.
NONIDEZ, JOSÉ F., Assistant Professor of Anatomy, Cornell University Medical College.
NORTHRUP, JOHN H., Member, Rockefeller Institute.
NOVIKOFF, ALEX B., Tutor, Brooklyn College.
ORR, PAUL R., Instructor, Brooklyn College.
OSTERHOUT, WINTHROP J. V., Member, Rockefeller Institute for Medical Research.

PACKARD, CHARLES, Assistant Professor, Institute of Cancer Research, Columbia University.
PARKER, G. H., Professor of Zoölogy, Emeritus, Harvard University.
PARPART, ARTHUR K., Assistant Professor of Physiology, Princeton University.
PATRICK, RUTH, Temple University.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
POND, SAMUEL E., Technical Manager, Marine Biological Laboratory.
PRESCOTT, GERALD W., Assistant Professor of Biology, Albion College.
PROSSER, C. LADD, Assistant Professor of Physiology, Clark University.
PUCKETT, WILLIAM O., Instructor in Biology, Princeton University.
PUMPHREY, RICHARD J., Rockefeller Fellow, Johnson Foundation, University of Pennsylvania.
RAMSEY, ROBERT W., Instructor in Zoölogy, University of Rochester.
RICE, KENNETH S., Woods Hole, Massachusetts.
RICHARDS, OSCAR W., Instructor in Biology, Yale University.
ROBERTSON, CHARLES W., Assistant Instructor, Washington Square College, New York University.
ROOT, WALTER S., Associate Professor of Physiology, Syracuse Medical School.
RUGH, ROBERTS, Instructor in Zoölogy, Hunter College.
SAMPSON, MYRA M., Professor of Biology and Head of Department, Smith College.
SANDOW, ALEXANDER, Instructor in Biology, Washington Square College, New York University.
SASLOW, GEORGE, Assistant Professor of Biology, Washington Square College, New York University.
SAYLES, LEONARD P., Assistant Professor of Biology, College of the City of New York.
SCHMIDT, IDA GENTHER, Instructor in Anatomy, College of Medicine, University of Cincinnati.
SCHMIDT, L. H., Research Fellow in Biochemistry, Christ Hospital and College of Medicine, University of Cincinnati.
SCHOTTÉ, OSCAR E., Assistant Professor of Biology, Amherst College.
SCHRADER, FRANZ, Professor of Zoölogy, Columbia University.
SCHRADER, SALLY HUGHES, Professor of Zoölogy, Sarah Lawrence College.
SCOTT, ALLAN C., Assistant in Zoölogy, Columbia University.
SHAPIRO, HERBERT, Research Council Fellow in the Biological Sciences, The University of Chicago.
SHAW, MYRTLE, Senior Bacteriologist, Division of Laboratories and Research, New York State Department of Health.
SHOUP, CHARLES S., Assistant Professor of Biology, Vanderbilt University.
SICHEL, FERDINAND J. M., Instructor, University of Pennsylvania.
SLIFER, ELEANOR H., Associate in Zoölogy, University of Iowa.
SMITH, CARL G., Demonstrator in Physiology, University of Toronto.
SMITH, DIETRICH C., Instructor in Physiology, College of Medicine, University of Tennessee.
SOLBERG, ARCHIE N., Assistant in Zoölogy, Columbia University.
SPEICHER, KATHRYN G., Pennsylvania College for Women.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia Medical School.
STEINBACH, HENRY B., Instructor in Zoölogy, University of Rochester Medical School.
STERN, CURT, Assistant Professor, Department of Zoölogy, University of Rochester.
STEWART, DOROTHY R., Assistant Professor of Biology, Skidmore College.
STIEHLER, ROBERT D., Research Fellow, Johns Hopkins University.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell Medical College.

STRONG, OLIVER S., Professor of Neurology and Neuro-Histology, College of Physicians and Surgeons, Columbia University.
STUNKARD, H. W., Professor of Biology, New York University.
STURTEVANT, A. H., Professor of Genetics, California Institute of Technology.
SUMMERS, FRANCIS M., Instructor in Biology, Bard College, Columbia University.
SUMWALT, MARGARET, Johns Hopkins University Medical School.
TASHIRO, SHIRO, Professor of Biochemistry, University of Cincinnati.
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Michigan.
TEORELL, E. TORSTEN, Docent in Physiological Chemistry, The Karolinska Institutet.
TEWINKEL, LOIS E., Assistant Professor of Zoölogy, Smith College.
TRAGER, WILLIAM, Assistant, Rockefeller Institute.
VICARI, EMILIA, Research Associate in Anatomy, Cornell University Medical College.
WATERMAN, ALLYN J., Assistant Professor of Biology, Williams College.
WEISS, PAUL, Assistant Professor of Zoölogy, The University of Chicago.
WHEDON, ARTHUR D., Professor of Zoölogy and Physiology and Head of Department, North Dakota Agricultural College.
WHITING, ANNA R., Professor, Head of Department of Biology, Pennsylvania College for Women.
WHITING, P. W., Visiting Investigator, Carnegie Institution of Washington, Cold Spring Harbor.
WILBRANDT, WALTER, Rockefeller Fellow, University of Pennsylvania.
WILLIER, B. H., Professor of Zoölogy and Head of Department, University of Rochester.
WILSON, EDMUND B., Da Costa Professor Emeritus in Residence, Columbia University.
WINTROBE, MAXWELL M., Associate in Medicine, Johns Hopkins University.
WODEHOUSE, R. P., Director of the Protein Laboratory, The Arlington Chemical Company.
WOLF, E. ALFRED, Assistant Professor of Zoölogy, University of Pittsburgh.
WOODRUFF, LORANDE L., Professor of Protozoölogy, Yale University.
WOODS, FARRIS H., Assistant Professor of Zoölogy, University of Missouri.
WOODWARD, ALVALYN E., Assistant Professor of Zoölogy, University of Michigan.
YOUNG, ROGER A., Assistant Professor, Howard University.
ZIRKLE, CONWAY, Associate Professor, University of Pennsylvania.

Beginning Investigators

ANGERER, C. A., Harrison Fellow, University of Pennsylvania.
BAKER, STANLEY, Instructor in Zoölogy, Wabash College.
BOSWORTH, MILLARD W., Dennison Research Assistant, Wesleyan University.
BULLOWA, ELIZABETH, Student, College of Physicians and Surgeons, Columbia University.
CHURNEY, LEON, Instructor in Zoölogy, University of Pennsylvania.
CLARK, JEAN M., Graduate Student, University of Pennsylvania.
COHEN, A. A., Harvard University.
COPELAND, D. EUGENE, Rochester University.
DEBOER, BENJAMIN, Graduate Assistant, University of Missouri.
DENNY, MARTHA, Student, Radcliffe College.
DERRICKSON, MARY B., Assistant, Vassar College.
DONNELLON, JAMES A., University of Pennsylvania.
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FARROW, JOHN, Graduate Student, University of Pennsylvania.
FRIEDMAN, SAM, Washington Square College, New York University.
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HAAR, FRANKLIN B., Graduate Student, University of Pittsburgh.
HALL, JOHN F., Assistant in Physiology, Princeton University.
HAWLEY, KATHARINE J., Student, Smith College.
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HOOVER, EARL, Johns Hopkins University.
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WICHTERMAN, RALPH, Graduate Student, University of Pennsylvania.
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ZUCK, ROBERT K., Oberlin College.

Research Assistants

ATCHLEY, DANA W., JR., Technician, Rockefeller Institute.
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BISHOP, DANIEL W., Swarthmore College.
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BRANDWEIN, PAUL F., Assistant, New York University.
BROWER, HELEN PORTER, Laboratory Assistant, Harvard University.
CARLSON, SVEN, University of Lund.
CLARK, JOHN K., Undergraduate Assistant, Trinity College.
COREY, H. IRENE, Research Assistant, University of Pennsylvania.
DEEHAN, SYLVESTER J., II, University of Pennsylvania.

FENNELL, RICHARD A., Assistant, Johns Hopkins University.
FISHER, KENNETH C., Fellow in the Department of Physiology, University of Toronto.
FLYNN, CARL M., Instructor in Zoölogy, University of Maine.
GLASSMAN, HAROLD N., Graduate Student, University of Pennsylvania.
GODRICH, JACK, Photographer, Columbia University.
GOFFIN, CATHERINE E., Research Assistant, Lilly Research Laboratories.
GOLDIN, ABRAHAM, Laboratory Assistant, Brooklyn College.
GRAND, C. G., Research Associate, Washington Square College, New York University.
HERSHKOWITZ, SOLOMON G., Columbia University.
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HÖBER, JOSEPHINE, University of Pennsylvania Medical School.
HOLMES, DOROTHY B., Assistant Bacteriologist, New York State Department of Health.
JOHNSON, JAMES B., Research Assistant, DePauw University.
KERSHAW, MARGARET A., Research Assistant, Wheaton College.
KOPPLEMAN, SAMUEL, University of Pennsylvania.
KRAATZ, CHARLES, Graduate Assistant in Zoology, University of Cincinnati.
LEHMAN, ELEANOR M., Assistant, University of Pittsburgh.
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RAWLES, MARY E., Research Assistant, University of Rochester.
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ROSE, SYLVAN M., Graduate Assistant, Amherst College.
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SASAKI, YASUO, Graduate Student, University of Cincinnati.
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STIX, HELEN D., Teaching Assistant, University of Cincinnati.
TAYLOR, JOHN F., Graduate Student, Johns Hopkins University.
THORNTON, CHARLES S., Assistant in Biology, Princeton University.
WARREN, MARSHALL R., Research Assistant, University of Cincinnati.
WILLIAMS, WILLIAM E., Student, Williams College.
WOHNUS, J. FRED, Assistant Instructor, Williams College.
YOUNG, SAUL B., Technician, Rockefeller Institute for Medical Research.

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BOTANY

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PEASE, ELEANOR F., Wellesley College.
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ZUCK, ROBERT K., Oberlin College.

EMBRYOLOGY

Aplington, Henry W., Student, Wesleyan University.
Becket, Ronald S., Student, Amherst College.
Bridgman, Jane, Smith College.
Brill, Edmund R., Undergraduate, Harvard University.
Copeland, Donald E., University of Rochester.
Dalton, Howard C., Undergraduate Assistant, Wesleyan University.
Dansereau, Antonio, Professor of Biology, Montreal University.
Engel, Jean, Pennsylvania College for Women.
Frank, Rhoda D., Hunter College.
Frothingham, Margaret, Sarah Lawrence College.
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Keister, Margaret L., Student Assistant, Wheaton College.
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Mapp, F. Everett, Teacher, Atlanta University.
Mowry, Helen A., Associate Professor of Biology, Skidmore College.
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Potsubay, Samuel F., Jr., Undergraduate Student, Amherst College.
Radi, Mohamed H., Member, Egyptian Educational Mission.
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Reinhard, Edward G., Associate Professor of Biology, St. Thomas College.
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Shaw, Roy C., Graduate Assistant, University of Rochester.
Smith, Jay Alfred, Laboratory Assistant, DePauw University.
Stewart, Walter A., Undergraduate Assistantship, Dartmouth College.
Taylor, Sarah P., Sarah Lawrence College.
Todd, Robert E., Austin Teaching Fellow, Harvard University.
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Zahl, Paul A., Teaching Fellow, Harvard University.

PHYSIOLOGY

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DeBoer, Benjamin, Graduate Assistant, University of Missouri.
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Evans, Eleanor M., University of Toronto.
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Greey, Elizabeth L., University of Toronto.
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Lippman, Richard W., Student, Yale University.

PAPPENHEIMER, JOHN R., Student, Harvard University.
ROBERTSON, KATHLEEN M., Research Fellow, University of Toronto.
SURRARRER, THOMAS C., Assistant Professor, Baldwin-Wallace College.
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WOODWARD, HUBERT E., Research Assistant, University of Toronto.

PROTOZOÖLOGY

ALBAUM, HARRY G., Student, Columbia University.
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CROWEN, EDWINA L., Columbia University.
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SCHOFER, ANNA, Goucher College.

INVERTEBRATE ZOÖLOGY

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BAUER, C. ADELE, Goucher College.
BAUMGARTNER, BARBARA, Butler University.
BERGREN, LESLIE, Instructor, University High School, Minneapolis.
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BRIGHT, WILLIAM M., Graduate Student, University of Illinois.
BROWN, RELIS B., Graduate Student, Yale University.
CAIRNS, JOHN MACKAY, JR., Hamilton College.
CASTLE, RUTH M., New Jersey State Teachers' College at Montclair.
CHAMBERS, WILLIAM N., Student, Amherst College.
CHILD, ESTHER W., Bennington College.
CLARK, BEATRICE, Wellesley College.
CONANT, BETSY D., Student, University of Rochester.
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DUGAL, LOUIS-PAUL, Professeur au Collège Ste.-Marie.
ENGLISH, JAMES E., Student, University of Missouri.
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GALAMBOS, ROBERT, Oberlin College.
GOODALE, MARION P., Teacher, Middlebury College.
GORDON, HAZEL E., University of Wisconsin.
HANSEN, DONALD F., Zoölogist, State Natural History Survey, University of Illinois.
HARPSTER, HILDA, Instructor, Sweet Briar College.
HATHAWAY, CHARLES O., JR., Graduate Student, Laboratory Instructor, University of Virginia.
HAYES, ELIZABETH A., Laboratory Assistant, Rockefeller Institute.

HENNING, WILLARD L., Graduate Assistant, University of Missouri.
HENSON, MARGARET, Smith College.
HEWITT, CORNELIA B., Smith College.
HOYT, DOROTHY, Student, Swarthmore College.
HUMMEL, ELIZABETH S., New Jersey College.
HUTCHENS, JOHN O., Undergraduate Assistant, Butler University.
JAKUS, MARIE A., Oberlin College.
LITWILLER, RAYMOND W., The University of Chicago.
MARTIN, WALTER E., Instructor in Zoölogy, Purdue University.
MATTOX, NORMAN T., Graduate Assistant in Zoölogy, University of Illinois.
MAYO, VIRGINIA, Graduate Assistant, Mount Holyoke College.
MCCONNELL, ELMA, New Jersey College.
MILLER, BLANCHE, Instructor, Agnes Scott College.
MOSELEY, RUSSELL L., Graduate Assistant, Wabash College.
PEASE, GWYNNETH, Laboratory Assistant, Wellesley College.
RENSHAW, BIRDSEY, Assistant, Harvard University.
ROBINSON, ROBERT A., Harvard University.
SCHMEICHEL, NORMAN L., Graduate Teaching Assistant, University of Wisconsin.
SCHROEDER, NANCY S., Sarah Lawrence College.
SHEPARD, CHARLES C., Wesleyan University.
SHETTLES, LANDRUM B., Graduate Student, Johns Hopkins University.
SNYDER, RUTH E., Laboratory Assistant, Barnard College.
STAUFFER, ROBERT C., Instructor in Biology, Dartmouth College.
WELSH, WILFRED R., Undergraduate Student, New Jersey State Teachers' College at Montclair.
WILDE, WALTER S., Teaching Assistant, University of Minnesota.
WILSON, JOHN WOODROW, Duke University.
WINTERNITZ, JANE K., Vassar College.
WISTAR, RAQUILITA, Wilson College.

3. TABULAR VIEW OF ATTENDANCE

	1931	1932	1933	1934	1935
INVESTIGATORS—Total	362	314	319	323	315
Independent	236	212	210	222	208
Under Instruction	83	73	66	49	56
Research Assistants	43	29	43	52	51
STUDENTS—Total	125	132	118	131	130
Zoölogy	55	55	54	54	55
Protozoölogy	17	16	11	11	16
Embryology	29	29	28	30	33
Physiology	17	18	19	23	20
Botany	7	14	6	13	6
TOTAL ATTENDANCE	487	446	437	454	445
Less Persons registered as both students and investigators	20	14	12	15	16
	467	432	425	439	429
INSTITUTIONS REPRESENTED—Total	137	141	120	131	143
By Investigators	102	94	92	98	111
By Students	68	76	58	75	70
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	–	–	1	1	–
By Students	4	1	2	5	3
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	8	8	5	4	7
By Students	1	1	–	1	1

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS IN 1935

Amherst College
Atlanta University
Barnard College
Berea College
Bowdoin College
Bryn Mawr College
Butler College
California Institute of Technology
Children's Hospital, Cincinnati, Ohio
Christ Hospital, Cincinnati, Ohio
College of Physicians & Surgeons
Columbia University
Cornell University
Cornell University Medical College
DePauw University
Duke University
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Iowa State College
Johns Hopkins University
Johnson C. Smith University
Eli Lilly & Co.
Lincoln University
Long Island University
Memorial Hospital, New York City
Morehouse College
Mount Holyoke College
New York State Department of Health
New York University
New York University Medical School
Northwestern University
Oberlin College
Pennsylvania College for Women
Princeton University
Purdue University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Royal Egyptian Legation
St. John's College
Seton Hall College
Smith College
Swarthmore College
Syracuse University
Temple University
Tufts College
Union College
University of Chicago
University of Chicago Medical School
University of Cincinnati
University of Illinois
University of Iowa
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Rochester Medical School
University of Vermont
University of Virginia
University of Wisconsin
Vanderbilt University
Vanderbilt University Medical School
Vassar College
Wabash College
Wellesley College
Wesleyan University
Western Reserve University
Wheaton College
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

5. EVENING LECTURES, 1935

Friday, June 28
Dr. W. J. V. Osterhout ..... "How Do Electrolytes Penetrate the Cell?"

Friday, July 5
Mr. Columbus Iselin ..... "The Current System of the Western North Atlantic."

Friday, July 12
Dr. E. F. Adolph ..... "Control of the Rate of Water Excretion in the Frog Kidney."

Friday, July 19
Dr. T. Hume Bissonnette ..... "Sexual Photoperiodicity."

Tuesday, July 23
Dr. Albert Navez ..... "Effects of Growth-promoting Substances in Plants."

Friday, July 26
Dr. Martin Kilpatrick ..... "Acid and Basic Catalysis."

Friday, August 2
Lt. Commander E. H. Smith .... "The Work of the International Ice Patrol as Carried Out by the United States Coast Guard."

Friday, August 9
Dr. Paul Weiss ..... "The So-called Resonance Principle of Nervous Control Revised."

Friday, August 16
(1) Dr. E. G. Conklin ..... "The History of Woods Hole and of the Marine Biological Laboratory."
(2) Dr. B. R. Coonfield ..... Motion Pictures Illustrating the Present Activities of the Marine Biological Laboratory."

Thursday, August 22
Dr. R. E. Cleland ..... "The Evening Primrose (Œnthera) —a Cytogenetic Non-conformist."

Friday, August 30
Dr. Hugh M. Smith ..... "Zoölogical Observations and Experiences during Twelve Years in Siam."

6. SHORTER SCIENTIFIC PAPERS, 1935

Tuesday, July 2
Dr. C. C. Speidel ..... "Effects of Alcohol on Nerves."
Dr. Roberts Rugh ..... "Ovulation in the Frog."

Tuesday, July 9
Dr. Dugald E. S. Brown ..... "The Liberation of Energy during the Simple Twitch of Skeletal Muscle."

Dr. W. Wilbrandt ..... "The Effect of Organic Ions on the Nerve Membrane Potential."

Dr. Kenneth C. Fisher and
Dr. Laurence Irving ..... "Respiratory Poisons and the Frequency of the Embryonic Fish Heart."

Dr. W. E. Garrey ..... "Respiratory Metabolism during Cardiac Inhibition."

Dr. Laurence Irving ..... "The Respiratory Metabolism of the Seal."

Tuesday, July 16

Dr. Ethel Browne Harvey ..... "Some Surface Phenomena in Centrifuged Sea Urchin Eggs."

Dr. D. P. Costello ..... "The Effects of Centrifuging the Eggs of Nudibranchs."

Dr. L. Monné ..... "The Permeability of the Nucleus of Amœba to Dyestuffs."

Dr. Robert Chambers ..... "Cortical Changes in Cell Division."

Tuesday, July 30

Dr. L. G. Barth ..... "Quantitative Studies on the Rate of Regeneration of Tubularia."

Dr. B. H. Willier,
Dr. R. F. Gallagher and
Dr. F. C. Koch ..... "Sex-modification in the Chick Embryo Resulting from Injections of Male and Female Sex-hormones."

Dr. Paul S. Galtsoff ..... "Physiology of Ovulation and Ejaculation in the Oyster."

Tuesday, August 6

Dr. A. K. Parpart ..... "The Permeability of the Erythrocyte to Heavy Water."

Dr. Oscar W. Richards ..... "Killing Organisms with Chromium as from Incompletely Washed Bichromate-sulfuric Cleaned Glassware."

Dr. C. V. Smythe ..... "The Effect of Oxygen Consumption on Alcoholic Fermentation in Yeast Extracts."

Dr. J. K. W. Ferguson ..... "The Scope of the Chemical Method of Estimating Hb-CO_2."

Dr. Shiro Tashiro ..... "Dacryorrhetin: the Demonstration of Its Action by a Motion Picture."

Tuesday, August 13

Dr. P. S. Henshaw ..... "Biological Factors Influencing the Radiosensitivity of Cells."

Dr. Henry J. Fry ..... "The Significance of Mid-bodies."

Dr. J. G. Carlson ..... "The Intergeneric Homology of a Euchromosome in Several Closely Related Acridinæ."

DR. C. E. MCCLUNG ..... "Phylogenetic Significance of Some Structural Conditions in the Orthoptera."

Tuesday, August 20

DR. WILLIAM TRAGER ..... "On the Nutritional Requirements of Mosquito Larvæ."

DR. J. E. DAVIS ..... "The Experimental Production of Tumors in Rats through Cellular Malnutrition."

DR. LEONARD B. CLARK ..... "Dark Adaptation in the Insect Dineutes assimilis."

DR. L. V. HEILBRUNN ..... "Protein Lipid Binding in Protoplasm."

DR. PAUL S. GALTSOFF ..... "The Effect of Crude Oil Pollution on Marine Life."

Tuesday, August 27

DR. H. BURR STEINBACH ..... "Diffusion Potentials in Biological Systems."

DR. W. J. V. OSTERHOUT ..... "The Rôle of Ions in Valonia and Nitella."

DR. M. H. JACOBS AND
DR. DOROTHY R. STEWART ..... "The Manner of Entrance of Ammonium Salts into Cells."

DR. E. TORSTEN TEORELL ..... "Some Aspects of Electrolyte Diffusion."

Thursday, August 29

DR. ROBERT CHAMBERS ..... "Studies on the Physical Properties of the Plasma Membrane."

DR. HOPE HIBBARD AND
DR. ROBERT CHAMBERS ..... "Micromanipulation of Egg and Nurse Cells in Bombyx mori."

DR. ETHEL BROWNE HARVEY ..... "Parthenogenetic Merogony or Cleavage without Nuclei in Arbacia punctulata."

MR. L. CHURNEY ..... "The Quantitative Determination of Mitotic Elongation."

DR. DONALD P. COSTELLO ..... "The Hyaline Zone of the Centrifuged Egg of Nereis."

MR. FLOYD MOSER ..... "Changes at the Surface of Arbacia punctulata Eggs during Membrane Elevation."

DR. HEINZ HOLTER ..... "Localization of Peptidase Activity in the Eggs of Echinarachnius and Arbacia."

DR. E. ALFRED WOLF AND
MR. T. F. MCBRIDE ..... "Early Stages of Ossification."

MR. T. F. MCBRIDE AND
DR. E. ALFRED WOLF ..... "Early Stages of Calcification in Teeth."

Dr. E. R. Clark,
Mrs. Eleanor Linton Clark and
Dr. R. O. Rex ..... "Observations on the Behavior of Polymorphonuclear Leucocytes as Seen in the Living Animal."

Dr. Elbert C. Cole ..... "Methylene Blue Staining Sequences in the Walls of the Digestive Tube of the Frog."

"The Effects of Ethyl Carbamate and of Potassium Cyanide upon the Staining Capacity of Ganglion Cells and of Cells of Smooth Muscle Type."

Dr. R. A. Budington ..... "The Tolerance of Acetyl-salicylic Acid by Sperm and Eggs of Arbacia."

Dr. Louise Palmer ..... "The Shedding Reaction in Arbacia."

Dr. H. H. Plough ..... "The Individual Specificity of Blood in Inhibiting Self-fertilization in Three Species of the Ascidian, Styela."

Dr. B. H. Grave and
Mr. Jay Smith ..... "Sex Inversion in Teredo and Its Relation to Sex Ratios."

Dr. Walter N. Hess ..... "Reactions to Light and the Photoreceptors in Dolichoglossus kowalevskyi."

Mr. James A. Donnellon ..... "An Experimental Study of Clot Formation in the Perivisceral Fluid of Arbacia."

Dr. Manton Copeland ..... "Keeping Nereis for Physiological Study."

Dr. Francis Leövey ..... "Changes in Osmotic Pressure of Teleost Muscle as a Result of Changes in External Salt Concentration (with special reference to the Tautog)."

Dr. E. F. Adolph ..... "Osmotic Exchanges of Phascolosoma."

Dr. W. A. Dreyer ..... "Water Content of Insects in Relation to Temperature and Humidity."

Mr. Millard W. Bosworth and
Dr. William R. Amberson ..... "An Adaptation of the Manometric Van Slyke Apparatus to the Study of the Respiration of Marine Animals."

Mr. John Stanbury and
Dr. William R. Amberson ....." The Rate of Regeneration of the Plasma Proteins after Complete Exsanguination."

Dr. Charlotte Haywood and
Dr. Rudolf Höber ....." The Permeability of the Frog Liver to Certain Lipoid Insoluble Substances."

Dr. M. E. Krahl and
Dr. G. H. A. Clowes ....." Effect of Nitrophenols and Related Compounds on Metabolism of Living Cells."

Dr. G. H. A. Clowes,
Dr. M. E. Krahl and
Miss Anna K. Keltch ....." Stimulation and Depression of Respiration in Relation to Cell Division."

Dr. A. E. Navez and
Dr. Ethel Browne Harvey ....." Indophenol Oxidase Activity in Intact and Fragmented Arbacia Eggs."

Miss Rita Guttman ....." Differential Oxygen Uptake of Regions of Limulus Optic Nerve as Related to Distance from the Sense Organ."

Dr. Walter S. Root ....." Lactic Acid in Dogfish Nerve."

Dr. Emil Bozler ....." Mechanical Properties of Smooth Muscles."

Dr. A. K. Parpart ....." A Method for Measuring the Intercellular Spaces in Tissues."

Dr. F. J. M. Sichel and
Dr. C. Ladd Prosser ....." Spatial Relations in the Excitation of the Isolated Muscle Fibre."

Dr. M. H. Jacobs and
Dr. A. K. Parpart ....." The Permeability of the Erythrocyte to Ammonia."

Mr. F. R. Hunter and
Dr. E. Newton Harvey ....." The Effect of Lack of Oxygen on the Permeability of the Egg of Arbacia to Ethylene Glycol." (Read by title.)

DEMONSTRATIONS

Thursday, August 29

Dr. Ethel Browne Harvey ....." Demonstration of Cleavage without Nuclei."

Mr. William E. Williams and
Dr. Elbert C. Cole ....." Permanent Preparations of Vertebrate and Invertebrate Tissues Stained with Phosphate-hæmatoxylin."

Friday, August 30

DR. EMIL BOZLER ..... "Significance of Calcium for Contractility of Vertebrate Smooth Muscle."

DR. E. R. CLARK AND MRS. ELEANOR LINTON CLARK .... "The Microscopic Study of Living Tissues in the Mammal."

DR. LEONARD B. CLARK ..... "Apparatus to Measure the Dark Adaptation and Intensity Discrimination of the Fiddler Crab as Related to the Visual Field."

DR. MANTON COPELAND ..... "A Tetrad for the Lecture Desk."

DR. HEINZ HOLTER ..... "Micro-titration Apparatus."

DR. LOUISE PALMER ..... "Shedding Reaction in Arbacia."

DR. A. K. PARPART ..... "Apparatus for Measuring Intercellular Fluid."

"A Method for Showing Swelling and Shrinking of Erythrocytes."

DR. ALEXANDER SANDOW ..... 'Diffraction Spectra of Striated Muscle."

DR. H. SHAPIRO ..... "Capillary Microrespirometer, after Gerard and Hartline."

DR. ELEANOR H. SLIFER ..... "One Hundred and Thirty New Organs in the Grasshopper."

DR. TORSTEN TEORELL ..... 'An Arrangement for Studying the Conditions within Diffusion Layers."

7. MEMBERS OF THE CORPORATION

1. LIFE MEMBERS

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ANDREWS, MRS. GWENDOLEN FOULKE, Baltimore, Maryland.

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FOOT, MISS KATHERINE, Care of Morgan Harjes Cie, Paris, France.

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THE MEMBRANES AND GERMINAL VESICLE OF THE EGG OF SABELLARIA VULGARIS

A. J. WATERMAN

(From the Thompson Biological Laboratory, Williams College, and the Marine Biological Laboratory, Woods Hole)

I

The newly-shed egg of *Sabellaria* is very irregular in shape and is enclosed by a rather thick vitelline membrane which is discontinuous with the egg protoplasm. Immediately after shedding this membrane elevates from the egg surface, the egg becomes spherical and, at the same time, a second or hyalin plasma membrane appears on the egg surface. Since the stimulus for membrane elevation seems to be the contact with sea water, the alterations undergone by these membranes during and after elevation have been examined in the light of those described for the membranes of other eggs after sperm entrance. The alterations restrict the time during which sperm penetration can take place.

A preliminary study has also been made of the free germinal vesicle of this egg. The vesicles can be secured within about eight minutes after shedding, by placing the eggs in distilled water. The behavior of the vesicles in hypo- and hypertonic sea water, in various salt solutions, and when exposed to certain vital dyes and indicators has been examined. The free vesicle does not go through any prematuration change; it will persist for hours in solutions which destroy the rest of the egg and sperm do not react to its presence.

II

Shedding is by way of the nephridiopores and is provoked by internal muscular activity, as well as by rhythmic contraction and elongation of the animal. After shedding, the contractions cease and the animal becomes quiescent.

As the eggs emerge from the nephridiopores they are of various shapes, caused by pressure in the coelomic cavities and during shedding; the endoplasmic granules are unevenly distributed; the germinal vesicle is large and somewhat irregular in outline; and in general the eggs appear quite light in color. Frequently the deformation is so pronounced that the wall of the germinal vesicle is in contact with that of

the egg. No trace of the original region of attachment of the egg to the ovary can be seen, nor is there any indication in the newly shed egg of the future animal pole. As the eggs emerge singly and the vitelline membrane is at first somewhat sticky, they adhere in long strings. Elevation and hardening of the membrane, rounding up of the eggs and movement of the female soon break up the strings and the eggs become loosely scattered on the bottom of the container.

After shedding several other changes can be seen to occur within the egg. As the eggs assume a spherical shape, the endoplasmic granules become more evenly distributed so that the eggs are no longer light in appearance, but are packed with granules which obscure the outline of the germinal vesicle. Because of the irregular shape of the eggs when first liberated, it has not been possible to determine if any change in volume occurs after shedding. The germinal vesicle becomes rounded and then irregular again and appears to move very slowly towards one side of the egg, the future animal pole. The irregular vesicle, or its derivative, continues this migration and eventually forms a clear, finely granular area, free from the larger granules, at the animal pole.

III

The Vitelline and Hyalin Plasma Membranes

Within the first few minutes after shedding the vitelline membrane elevates from the egg surface. At first the elevation is quite uniform and the membrane appears smooth and without wrinkles, but as elevation proceeds it becomes irregular and abundantly wrinkled. This may be the result of differences in tension of the protoplasmic processes. The deeper indentations may extend to the egg surface (Fig. 1). This state persists generally for a long time and it is only in very old eggs or after fertilization when these processes are withdrawn, that the wrinkles disappear. The outline of the membrane then becomes slightly oval rather than spherical and the space between membrane and egg is enlarged.

Coincident with the elevation of the vitelline membrane, a thin layer of transparent hyalin material of uniform thickness, the hyalin plasma membrane, appears on the surface of the egg. From the egg surface, numerous fine protoplasmic strands or threads can be seen to extend towards the vitelline membrane and they have been traced to the membrane itself. These persist until fertilization, and may be concerned in transmitting the stimulus of sperm contact across the subvitelline space. They disappear following centrifuging. It remains to be seen

if lightly centrifuged eggs from which the strands have disappeared can be fertilized. The few preliminary experiments of this kind have not been successful. Hobson (1932) describes protoplasmic strands in the egg of *Teredo norvegica* which are pulled out from the egg surface during the separation of the vitelline membrane. These may be slender threads or broad flame-shaped processes. This is followed by the slow retraction of the strands which seem as though composed of a viscous fluid. If a strand breaks during retraction each half

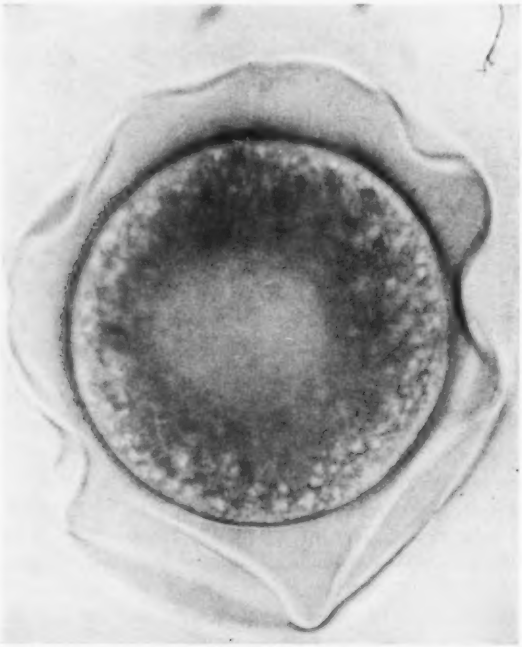

FIG. 1. Photomicrograph of a newly-shed egg. The elevated wrinkled vitelline membrane, the fine protoplasmic strands extending from the egg surface towards the membrane, and the extension of the germinal vesicle material toward one side of the egg are visible.

contracts, one to the egg surface where it disappears and the other to the membrane surface.

In *Sabellaria* the strands are likewise of a viscous nature, but they are very fine and numerous and appear to be pulled out by the elevation of the vitelline membrane (Fig. 1). The material of the hyalin plasma layer seems to form between and around their bases. The

strands are more permanent than those described by Hobson. They persist for hours in old eggs and withdrawal occurs after sperm entrance, centrifuging, treatment with hypotonic sea water or distilled water, etc. During retraction following fertilization, they do not break but become shorter, thicker, beaded or knobbed and finally disappear within the egg surface. The strands are evidently under some tension and the material of which they are composed is elastic. After their withdrawal the hyalin plasma membrane becomes a uniform layer. During the elevation of the fertilization cone, the strands involved become lost in the substance of the cone. No separate fertilization membrane appears after sperm entrance.

The vitelline membrane of the sabellarian egg appears to be a preformed structure (*cf.*, Fauré-Fremiet, 1924). In stained sections of the female, it is visible as a very thin but distinct membrane enclosing the unshed eggs as they lie in the coelomic cavity. It may also be seen to encircle the developing oöcytes except where they are attached to the ovary. At this time the membrane is thin but gradually it increases in thickness as development proceeds. It seems to be discontinuous with the egg cytoplasm. In freshly-shed eggs, it is slightly thicker as some swelling occurs on contact with the sea water, but during elevation it becomes thinner as it is stretched. At first the membrane is soft, somewhat gelatinous, elastic and slightly sticky. Tension cannot be very great or the eggs would approach more towards a spherical shape while in the abdominal cavity of the female.

The plasticity of the membrane changes after exposure to sea water. The change in the shape of the egg is a surface phenomenon involving a permeability change and is not influenced directly by the vitelline membrane, though if elevation is retarded, the rounding up of the eggs takes place more slowly. Elevation occurs simultaneously and in a somewhat uniform manner over the entire egg surface, not starting at one point (sperm contact-point) as is the case with fertilization membranes. During fertilization the force of sperm entrance may pull the membrane towards the egg, but after the sperm-head is through, it flips outward again to its original position.

After contact with sea water, the vitelline membrane hardens, as shown by placing the eggs at different times after shedding into distilled water and noting the rate and the numbers of the eggs which swell and cytolyze accompanied by further elevation and rupture of the membrane (see Moore, 1930; Hobson, 1927; and Costello, 1935 for fertilization membranes). For example, eggs shed from 2:24–2:25 P.M. were transferred at one-minute intervals to plain distilled water. At this time the eggs were spherical and the membrane had elevated.

In eggs transferred within the first five minutes cytolysis occurred immediately and the membrane elevated until it broke. It quickly vanished and the cytoplasm, except for the vitelline granules, disappeared leaving naked swollen germinal vesicles. In eggs transferred during the next seven minutes a progressively increasing number of the membranes failed to rupture immediately, and they persisted for longer intervals. The membranes of eggs transferred at 2:38 persisted without rupture or other change for hours.

This, together with other experimental data, indicates a pronounced hardening of the vitelline membrane with loss of solubility and elasticity during the first twelve minutes after shedding. The rate of cytolysis was much the same in the several cultures, showing that there was little change in the permeability of the egg and membrane to water during the period. The amount of hardening during the first hour at least is not sufficient to inhibit sperm entrance, but there may be some retarding effect since the highest percentage of fertilization has been secured when eggs and sperm were shed together in the same container. At the time of shedding the membrane is quite soft and, as seen above, it is readily soluble in distilled water. This physical change in the membrane may account in part for the very low percentage of fertilization secured in eggs which have stood for longer than one hour.

The membranes which persist for some time after escape of the contents are irregular in outline and markedly thinner than those of normal eggs. This shows that the preformed membrane is capable of considerable extension in newly-shed eggs, as has been demonstrated by Just (1928) for the unfertilized egg of *Arbacia* and by Chase (1935) for the egg of *Dendraster*. Moreover, the membranes become rigid, fail to collapse and the ruptured edges do not curl or stick to the membranes of adjacent eggs with which they come in contact.

The physical changes occurring in the hyalin plasma membrane, following its first appearance, resemble in several respects those of the vitelline membrane. At first it is thin, gelatinous, and quite soft, offering no resistance to the swelling and bursting of the egg in distilled water. It is weaker than at a later stage. After the elevation of the vitelline membrane, the plasma membrane increases in thickness. Since it is not visible before elevation and its appearance is not dependent on fertilization, it may be the result of the reaction of the exposed cortical cytoplasm of the egg. Within one-half hour after shedding, there is a noticeable stiffening and loss of elasticity on the part of the membrane. It slowly becomes fairly tough, rigid and less soluble, but not to the same extent nor as quickly as does the vitelline membrane. Because of this alteration a longer time is required for the eggs to burst or they may

not burst at all but form exovations. This progressive hardening of the hyalin plasma membrane continues slowly. When tested with distilled water the older eggs cytolyze quickly, swell slightly, but only a few burst even after several hours of exposure.

Harvey (1934) describes the hyalin plasma membrane of fertilized sea-urchin eggs as a thin colorless layer which appears on the surface of the egg after fertilization. Ten to fifteen minutes after fertilization it thickens. It is an extracellular membrane rather than an integral part of the cell protoplasm. The observations of Harvey would seem to support the conclusion that the appearance of the plasma membrane coincides with the elevation of the vitelline membrane irrespective of what initiates the elevation of the latter. Moore (1928) considers it to be a secretion membrane. He finds that the material, of which this membrane is made, is secreted by the fertilized egg, blastomeres, blastulæ and early gastrulæ, but not by advanced gastrulæ and plutei. The addition of Ca to sea water renders this membrane relatively insoluble and inelastic.

Unlike the results of Moore (1935) in the case of the eggs of certain echinoids, isosmotic glycerine and urea (pH close to that of sea water) do not seem to inhibit membrane elevation in freshly shed eggs. Instead there is an acceleration of elevation in these non-electrolyte solutions and the membrane rapidly disappears. When returned to fresh sea water, the eggs which did not cytolyze and disintegrate underwent the prematuration changes of normal unfertilized eggs. Eggs freed of their vitelline membranes by the above method exhibited marked constancy of form and size in fresh sea water, as revealed by camera lucida outline drawings at frequent intervals. No processes were present.

These eggs are capable of being fertilized. No elevation of any secondary membrane was seen to follow sperm entrance. The cleavages were typical as far as they were followed and the blastomeres formed the compact cellular mass characteristic of normal development. From the above observations it is difficult to imagine any other function for the vitelline membrane of this animal egg than that of protection to the developing embryo. Its early elevation forms no obstruction to sperm entrance, possibly aided by the presence of protoplasmic processes which traverse the subvitelline space and transmit the stimulus of sperm contact. The absence of these processes in membraneless eggs offers no obstruction to fertilization.

In other experiments, eggs shed into isosmotic glycerine (pH about 7.0) were transferred at one-minute intervals thereafter to fresh sea water. The eggs returned to normal size and membranes elevated in cultures transferred up to five minutes after shedding. The numbers

elevating membranes steadily decreased during this interval. Eggs transferred after five minutes exposure formed no membranes during the course of an hour. The remaining eggs in the glycerine were transferred after thirteen minutes exposure to distilled water. Entire disintegration occurred, except in the case of the germinal vesicles. A more acid glycerine solution, with a pH of about 6.0, gave different results from those just described. The membranes elevated as before, though a trifle more slowly, and instead of disappearing, persisted for about an hour. Thus a more acid glycerine preserves the vitelline membrane for some time but does not prevent its elevation. In general eggs shed into glycerine show a marked tendency to clump or stick together.

Chase (1935) has studied the solubility of egg membranes in an isosmotic solution of urea. After such treatment the unfertilized eggs of *Dendraster excentricus* and *Strongylocentrotus purpuratus* failed to form membranes and the fertilization membrane in the early stages of its formation also was soluble. Both the vitelline and fertilization membranes of *Patiria miniata* and *Urechis caupo* were insoluble but those of the latter could be dissolved off after fifteen to twenty-four hours exposure. In the present study the membranes elevated immediately before the glycerine had time to dissolve them. Afterwards they were dissolved but the time required increased with the age of the eggs.

The effect of ions upon the elevation of the vitelline membrane in glycerine was tested by adding varying amounts of M/60 $CaCl_2$, M/60 $MgCl_2$, M/40 NaCl or M/40 KCl to the glycerine. The eggs from different females were used for the study, and the eggs were shed directly into the solutions. The addition of $CaCl_2$ to the glycerine preserves the vitelline membrane and also retards its elevation. Potassium chloride accelerates membrane elevation while $MgCl_2$ retards elevation in the higher dilutions. The latter also retards rounding up of newly-shed eggs and the eggs clump together in large masses. Sodium chloride seems to have an accelerating effect upon membrane elevation but rounding up of the eggs is retarded. The few eggs which cytolyzed and burst, liberated free germinal vesicles which remained intact for hours in the solution.

The relative effect of these chlorides parallels that found by Lucké and McCutcheon (1932) in their investigation of the effect of ions upon the water permeability of sea-urchin eggs. When dissolved in a hypotonic solution of dextrose, the chlorides of Na, K, Li, or NH_3 increased the permeability to water more than in pure dextrose. When the chlorides of Ca or Mg were used, the permeability decreased to a point near that of sea water. According to Fauré-Fremiet (1924) the

vitelline membrane of the egg of *Sabellaria alveolata* is permeable to electrolytes, crystalloids and dyes. Its elevation is not due to the osmotic pressure of the peri-ovular fluid but to the fact that the electric charge on the inner face of the membrane is the same as that on the surface of the egg. After the elevation of the vitelline membrane, the spherical egg is without any trace of another membrane.

IV

Germinal Vesicle

The vesicle of the newly-shed egg is a large, clear, irregular-shaped structure containing a single eccentrically located nucleolus and a few large granules in a fluid, finely granular nucleoplasm. No precise structure can be seen in it even when vitally stained. As the egg becomes spherical, the vesicle also rounds up but the distinctness of its outline is lost by the more uniform distribution of endoplasmic granules and globules. Shortly after this, it loses its spherical outline and the vesicle or its derivatives move toward one side of the spherical egg where a finely granular area appears at the animal pole. During this migration the outline of the vesicle undergoes a variety of changes in shape as shown by camera lucida outline drawings of individual eggs.

Any substance which produces cytolysis and sufficient swelling so that the egg membranes burst will give free naked germinal vesicles during a certain time after shedding, e.g., distilled water, hypotonic sea water and glycerine. In these solutions, swelling of the germinal vesicle occurs during cytolysis and swelling of the egg but not before. The osmotic equilibrium established at this time does not change even after long exposure to plain distilled water. There is little increase in size or other change after the swollen vesicles are freed from the remainder of the egg material and they remain intact for hours in hypertonic and hypotonic solutions, sea water, glycerine or distilled water. When transferred from distilled water to sea water or to a hypertonic solution, they shrink.

Several observations seem to point to the presence of an elastic solid surface or membrane in the free germinal vesicle which is in a state of tension. During and after swelling in distilled water the vesicle is spherical. The elastic solid layer is stretched but its elasticity is limited since occasionally it may rupture. The exovates are not soluble. In this case the vesicle proper decreases slightly as material is extruded, but the surface does not wrinkle. No thick structure or membrane surrounds the exovates as is the case with the vesicle itself.

In hypertonic sea water the free vesicle shrinks uniformly and with

a smooth surface to about the normal diameter. With continued exposure it becomes crenated or even indented and with time this condition becomes more marked. Thus when water is removed by a hypertonic environment, the surface layer contracts, after which reduction of volume results merely in puckering or wrinkling. This stretching is not always reversible and the amount of contraction is variable in different vesicles from the same female. The layer contracts more in hypertonic salt solution than in sea water. Many swollen vesicles show little reduction in size when transferred to sea water, while others return to about normal size and do not pucker.

During swelling the vesicles practically double the original diameter but do not generally rupture no matter how long they are subsequently left in plain distilled water. They have been found twenty-four hours later with practically no change except for a clumping of the larger granules and globules at one side in the region of the nucleolus. Thus the surface layer as well as the contents of the vesicle, i.e., exovates, are insoluble, and this layer is quite tough, as swollen vesicles do not generally burst. Another observation is that vesicles, which have shrunk on transference to sea water and after an interval are placed again in distilled water, do not swell again or swell very little without rupture. This would seem to indicate a hardening and loss of elasticity of the surface layer after some exposure to sea water.

Fauré-Fremiet describes the germinal vesicle of *Sabellaria alveolata* as being about 55 μ. The contents are quite aqueous and a single acidophilic nucleolus is present. The germinal vesicle swells in strong concentrations of NaCl and $MgCl_2$, and, if the egg bursts, the vesicle is freed and will persist for many hours.

The free vesicle shows no specific internal structure with vital dyes. A large persistent nucleolus is present as well as a small number of large, irregular-shaped granules of various sizes. The ground substance seems to be a finely granular material. No evidence of any prematuration changes have been seen in the free vesicle even when transferred to sea water immediately after escaping from the cytolyzed egg. Vesicles which have stood in the solutions for some time, for example distilled water, show less reduction in size when returned to fresh sea water than those which are transferred soon after rupturing. It has been noticed that swelling of the vesicle accompanies egg swelling and cytolysis. Eggs which are slow to react to distilled water or glycerine do not show any preliminary enlargement or change in shape of the vesicle.

Free germinal vesicles can be secured from eggs up to about nine minutes after shedding. The greatest number appear from eggs shed

directly into distilled water or placed in it during one to four minutes after shedding. Prematuration changes start around seven to eight minutes after shedding, so the vesicles secured after this time represent those which were somewhat delayed. In isosmotic glycerine, prematuration does not take place and the vesicles persist. If rupture does not occur here, free vesicles can be secured by transfer of these eggs to distilled water.

The wall of the germinal vesicle is permeable not only to water and perhaps glycerine, but also to certain dyes. The dyes employed include methyl violet, orange G, acid fuchsin, chrysoidin, methyl red, neutral red, methyl green, methylene blue, nile blue sulphate, and indicators as brom thymol blue, cresol red, thymol blue, and phenolphthalein. The germinal vesicles were transferred immediately after their liberation to fresh sea water and a dilute solution of the dye added.

Methyl violet in distilled water penetrated the isolated vesicle at once and gave a faint pinkish violet, with a pH above 5. Some of these eventually formed exovates which were stained, showing that the dye penetrated the vesicle wall. The cytolyzed egg cytoplasm stained a blue violet color, at a pH between 2.0 and 5.0. When transferred to sea water the vesicles became a light violet. The cytoplasm of normal unfertilized eggs of the same age were colored a deep blue violet which, together with evidence from other dyes and indicators, shows a pH above 5.0 and probably around the neutral point for the living egg. After transfer to distilled water, these eggs ruptured but the free germinal vesicle was without any trace of color. The depth of color of the free vesicle varies slightly with the concentration of the dye. Those in fresh sea water color a light violet, indicating a pH of 6.0 or above. The pH of sea water was around 8.2 and that of the distilled water 5.8. This dye stains the vitelline membrane a light pinkish violet.

In distilled water brom thymol blue colored the vesicle a light yellow, pH not above 6.0, while in sea water the color was a yellowish blue, showing a pH value between 6.0 and 7.0. These results would seem to indicate a variation of the internal pH of the free vesicle in harmony with that of the external medium. Orange G, acid fuchsin, and chrysoidin gave little, if any, differential staining. Methyl red stained the vesicle in sea water a yellowish red, indicating an internal pH around 6.0. Methylene blue is rapidly reduced by the vesicle.

Neutral red seemed to penetrate the vesicle quite slowly. It showed a densely granular structure with irregular clumping of the larger granules and globules. The red color indicated a pH below 7.0. This dye also penetrated the vitelline membrane slowly. Methyl green and methylene blue stained the granules. Nile blue sulphate entered read-

ily and immediately gave a dark blue color even when noncytolyzed eggs showed no coloration. The dye seemed to enter with equal facility and to almost the same degree whether distilled water or fresh sea water constituted the external medium. Intact but cytolyzed eggs did not stain during the hour of observation.

In neutral red, methyl violet, methyl red and Nile blue sulphate, both the wall and contents of the germinal vesicle are stained. This is shown by the many cases in which colored exovations formed after the vesicle had stood for some time. Neutral red penetrated the vesicle membrane more slowly than any of the dyes employed. After an hour they were only slightly colored.

The above experiments represent only an initial study of the action of dyes on the free germinal vesicle. They would seem to indicate an internal pH value of the free germinal vesicle in fresh sea water between 6.0 and 7.0. In distilled water and sea water which are acid, the pH of the vesicle tends toward the acid side. In neutral distilled water or sea water the pH is near the neutral point. It is possible that these results have no real significance because the vesicle is not in its normal environment and furthermore it may be fatally injured by the method used in freeing the vesicle. If the phenomenon of cytolysis can be said to occur in the germinal vesicle freed by distilled water, then it must be expected (Needham, 1931) that it would appear more acid than is normally the case.

Needham (1931), in the discussion of the action of vital dyes on whole eggs, considers that the intracellular reaction is near the neutral point and that cytolysis is an acid-producing process. Injection of neutral red into the egg of *Sabellaria alveolata* gave a pH of 6.6. For this same egg Fauré-Fremiet (1924) found a pH less than 7.0 at zero to five minutes after shedding. This gradually increased to 12.0 or higher at forty to fifty minutes after shedding. If fertilized, there was an increase to 10.4 or 11 and then a fall to around 7.0 during the first stages of segmentation. Needham criticises these results as given by dyes which are not good pH indicators even under the best of conditions and which could not be trusted in a medium containing fatty substances for which they have a special affinity.

Needham also gives the results of Reiss, who found that in the unripe eggs of *Sabellaria:* (1) bromcresol purple gave a deep mauve for the germinal vesicle and yellow for the cytoplasm, and (2) bromthymol blue gave yellow for the cytoplasm, yellowish green for the germinal vesicle and bluish green for the nucleolus. The micro-compression method gave a pH of 5.0 for *Sabellaria* but this might be the result of injury (Needham). Like the results just described, he found

that the pH of the nucleus was markedly affected by the pH of the external medium and varied in harmony with it. The pH of the intact vesicle, as found by Reiss (*cf.*, Needham, 1931), is very slightly higher than that found in the case of the isolated vesicle which was more toward the acid side.

In the present study it was noticed that the dye penetrated the germinal vesicle if it was free, but the vesicle of intact normal eggs did not generally stain during the experimental period, since after rupturing, the vesicles of stained eggs were colorless. The vesicle of cytolyzed and swollen eggs did stain. Monné (1935) has studied the permeability of the nuclear membrane of two *Amœba* species to vital stains. He used some forty dyes and sprayed them against the nuclear membrane with a micropipette. Immersion of the whole *Amœba* in the dyes failed. The living cytoplasm of the normal sabellarian egg, as well as that of the *Amœba,* acts as a barrier to dye penetration into the nucleus when sublethal concentrations of the dye are used. If the egg is killed, then certain dyes will enter and stain diffusely.

Summary

The vitelline membrane of the egg of *Sabellaria vulgaris* is a preformed structure whose elevation is stimulated by exposure to sea water. During and after its elevation a second or hyalin plasma membrane forms. During the first hour after shedding these membranes undergo certain physical alterations which probably are responsible in part for the very low percentage of successful sperm penetration. These alterations are described as well as the effect of a non-electrolyte solution and various salts on membrane elevation.

The behavior of the free germinal vesicle of this egg in sea water, hypo- and hypertonic solutions is also described. The vesicle, freed of any of the other egg materials, can be secured within about eight minutes after shedding, by placing the eggs in distilled water. The penetration of certain vital dyes and indicators is discussed and also the information these reveal of the internal pH of the free vesicle.

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ON THE ENERGETICS OF DIFFERENTIATION. III

Comparison of the Temperature Coefficients for Cleavage and Later Stages in the Development of the Eggs of some Marine Animals

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These experiments show principally that the temperature coefficients of the rate of attainment of the various cleavage stages and of the later developmental stages are identical for the eggs of some marine animals.

Theoretical Part

It has been concluded from earlier work (Tyler, 1933, 1935) that energy is required for the processes of embryonic differentiation, although the quantities involved could not be estimated. As part of a program to evaluate the energy requirements, an investigation of the temperature coefficients of the rate of development and of the rate of respiration was undertaken. This involved the determination of the temperature coefficients for cleavage for comparison with that of differentiation. In the previous work distinction was made between the maintenance, growth and differentiation processes of development. Cell division was left out of account. This was justified inasmuch as it did not enter as a factor in the results. In the cases of both the "half-embryos" and the giant embryos gastrulation occurs when the original egg has undergone the same number of cell divisions as it normally does. This was shown in the early work of Morgan (1901, 1903), Driesch (1900) and Bierens de Haan (1913). In considering the effect of temperature on development there is, of course, the possibility that cleavage and differentiation may be affected differently. That would mean that the embryos in the same stage of differentiation would contain different numbers of cells if reared at different temperatures, provided, of course, that the later divisions have the same temperature coefficient as the earlier ones.

The data that exist in the literature in regard to this point are of two sorts, namely, that in which estimates of the number of cells present in embryos of the same stage, but raised at different temperatures, are

[1] I am indebted to Mr. W. D. Humason and Mr. C.-C. Tan for collecting some of the data presented here and to Professor T. H. Morgan for his criticism and suggestions.

given, and that in which the temperature coefficients of the rate of cleavage and of attainment of the later stages are given. Data of the first kind are found in the work of Marcus (1906), Godlewski (1908), Boring (1909) and Koehler (1912), all of whom were interested in the effect of temperature on the nucleo-plasma ratio. The second kind of data is found in the work of Hertwig (1898), Peter (1905), Erdmann (1908), Krogh (1914), Ephrussi (1933) and Atlas (1935). With the exception of Boring, all of the first group find different numbers of cells in embryos of the same stage raised at different temperatures. In the second group only Atlas finds the same values for the temperature coefficients of the rate of cleavage and of attainment of the later stages.

Marcus, Godlewski and Koehler worked with the sea urchin, and although they all found larger numbers of cells in the embryos of the same stage raised at the higher temperatures, the relative values differed rather widely. Thus Marcus found the ratio of the number of cells in blastulæ at the beginning of mesenchyme formation to be 1 : 2.5 for the temperatures 9° and 22° C.; Godlewski obtained 1 : 1.81 for 12° and 21°; Koehler obtained 1 : 1.11 for 10° and 22°. Koehler claimed that the method employed by Marcus and Godlewski of counting the nuclei in the optical section was less accurate than his own method of counting the nuclei in a definite area of the surface of the embryo. Although he obtains values that are fairly close for the number of cells in blastulæ raised at different temperatures, in the later stages the differences are more marked. His figures for the early and late gastrula stages are:

Early gastrula	624 (10°),	820 (16°),	869 (22°)
Late gastrula	1040 (10°),	1163 (16°),	1595 (22°).

It may be noted that in the early gastrula stage the cell numbers for 16° and 22° are much closer than for 10° and 16°, whereas in the late gastrula the reverse is true. This it seems reflects on the accuracy of the results.

Miss Boring worked on the eggs of *Ascaris*. Her data are not very extensive, the counts being limited to groups of 3 to 5 cells of the surface ectoderm of the head region of embryos raised at 18° and 37° C. But she concludes that the number of cells is the same in embryos raised at the two different temperatures. The criticism of Erdmann (1908*b*) that the range of temperatures employed is outside of that for normal development cannot be significant, since Zawadowsky and Sidorov (1928) found that *Ascaris* eggs could develop at 7° C.

The temperature coefficient measurements of Peter gave higher values for cleavage than for the later stages in the case of the sea urchins, *Sphærechinus* and *Echinus*. Peter also calculated the co-

efficients for the frog from Hertwig's data and in this case the values for cleavage were lower than for the later stages. His Q_{10} figures are

Cleavage	2.29 (*Sphærechinus*)	2.30 (*Echinus*)	2.23 (*Rana*)
Later stages	2.03 (*Sphærechinus*)	2.08 (*Echinus*)	3.34 (*Rana*)

However, one can hardly attribute much significance to these figures since they are averages of values from widely different parts of the temperature scale, and as Peter himself shows, the Q_{10} at low temperatures is much higher than at high temperatures. Moreover, constant temperatures were not maintained, so that it is fruitless to try to compare the data for the later stages with cleavage even in a single experiment.

Krogh investigated the frog's egg at temperatures ranging from 2.7° to 22°. He chose five stages in the later development from closing of the medullary folds to the 7.8 mm. larva and found that the rates of attainment of these stages were similarly affected by temperature. He did not compare the values for the later stages with those for cleavage, but from his curves it can be seen that in the interval of 10° to 20° C. cleavage is speeded up 2.4 times, whereas the figure for the later stages is 3.6.

Ephrussi determined the time of first cleavage and of hatching of the blastula out of the fertilization membrane for the sea urchin at temperatures between 10° and 26° C. From his graphs (p. 85) it may be seen that cleavage is speeded up 3.1 times at 20° as compared with 10° and the rate of attainment of the hatching stage is speeded up 3.0 times. These values are evidently the same, but he draws the opposite conclusion from the data as a whole. Ephrussi considers hatching to represent a definite morphological stage (*cf.* p. 120). This is, however, not necessarily the case. In the trout embryo Gray (1928) finds that the morphological development at the time of hatching is different with different temperatures of incubation. My own experiments with *Ciona* show that embryos raised in concentrated cultures hatch out sooner than those in more dilute cultures. Thus for two cultures raised at 15° C., one containing about five hundred thousand embryos in 50 cc. of sea water, and the other about five thousand embryos in 50 cc., the following figures were obtained for the percentages of the embryos hatched at the indicated times after fertilization:

	Hours after fertilization						
	24.0	24.4	24.7	25.0	25.4	25.7	26
Concentrated culture	10	40	70	95	100	100	100
Dilute culture	0	0	0	10	40	80	95

The rate of cell division, however, was quite the same in both cultures. The earlier hatching of the crowded culture is very likely due to a greater concentration of the hatching enzyme which Berrill (1929) has shown to be produced by ascidian embryos.

Recently Atlas (1935) has found the temperature coefficients for the later stages of development of the frog's egg to be the same as for the cleavage stages over a considerable range of temperatures. Divergence began to appear above 20° C. but below that temperature the deviations are apparently within the limits of variability of the material.

It is evident from the lack of general agreement that further investigation of these points is desirable.

Experimental Part

Material and Methods

In these experiments the eggs of five marine animals were used; namely, the sand dollar, *Dendraster excentricus*, the sea urchins, *Strongylocentrotus purpuratus* and *Lytechinus anamesus*, the ascidian, *Ciona intestinalis*, and the gephyrean worm, *Urechis caupo*. Only batches of eggs giving better than 95 per cent fertilization were followed, since there is the possibility that such factors as are responsible for the failure of a large percentage of the eggs to become activated may also affect the rate of development of the fertilized eggs. Also the disintegrating unfertilized eggs might at later stages affect the development of the others. In the great majority of the experiments, however, 100 per cent fertilization and 95 to 100 per cent normal development was obtained.

The temperature was controlled by means of a mercury-toluene thermoregulator operating through an a.-c. vacuum tube relay, and the variations were less than 0.01° C. For temperatures below that of the room, cold water was pumped from a refrigerated bath through coils of copper tubing in the constant temperature bath. This method was more satisfactory than direct immersion of the expansion coils of the refrigerator in the temperature bath, since the flow of cold water through the coils could be regulated so that it approximately balanced the warming-up of the bath.

The eggs were raised in flasks that were continuously shaken in the temperature baths. The rate of shaking was generally 50 round-trips per minute and the amplitude 3.5 cm. The flasks used were 125 cc. Erlenmeyers. They were filled with 50 to 60 cc. of sea water and contained in most runs a volume of eggs of about 5 cu. mm.

Two temperature baths were employed, so only two temperatures could be investigated at one time. The temperature was changed in

only one bath at a time for new runs, leaving one to check any differences due to using eggs from different animals. Thus comparable data for all the temperatures employed could be obtained and the temperature-rate of development curves of Figs. 3 and 4 could be made. However, to compare the effects of temperature on the rate of cleavage and on the rate of attainment of the later stages it is sufficient to operate at two different temperatures at one time.

The eggs were either fertilized while in the temperature baths or introduced into the flasks in the baths at a definite time after fertilization. Since the flasks in the lower temperature baths would warm up temporarily upon the addition of an egg or sperm suspension of higher temperature, an appropriate amount of sea water cooled to the proper extent was, when necessary, added at the same time. This precaution is, of course, unnecessary except in cases of very low temperatures and when a large volume of sperm or egg suspension, in comparison with the volume of sea water in the flask, is added.

The time of attainment of a definite stage was determined by removing samples from the flasks at frequent intervals of time and either examining them immediately or preserving them for detailed examination. The time when 50 per cent of the developing eggs have reached a particular stage is given in the data, unless otherwise specified. For the cleavage stages the end-point chosen is that point at which the constriction of the cell first appears, for the later stages the end-points chosen are given in data presented.

The distribution curves for the attainment of a particular stage are not the same in relative magnitudes for the eggs of the various animals investigated. The eggs of *Ciona intestinalis* behaved most uniformly. As an example, the percentage of divided eggs found at various times after fertilization for a batch of eggs at 20° C. may be given.

	Minutes after fertilization					
	46¼	46½	46¾	47	47¼	47½
Per cent in 2 cells.....	0	10	20	50	85	100

The time difference between the first and last egg to divide is less than one minute for an average time of 47 minutes from fertilization. With the eggs of *Dendraster excentricus* the time difference was relatively greater as the following figures for a typical batch of eggs run at 20° C. show.

	Minutes after fertilization						
	53½	54	54½	55	55½	56	56¼
Per cent in 2 cells.....	0	5	20	50	75	95	100

The spread here is about 2¼ minutes for an average time of 55 minutes. *Urechis caupo* eggs gave a spread of about 2½ minutes for a first cleavage time of 76 minutes. *Strongylocentrotus purpuratus* eggs gave a 4-minute spread for a cleavage time of 77 minutes. For *Lytechinus* the figures at 15° are 7 and 137 minutes. At different temperatures the relative spread of the distribution curves will be the same since the time difference between the first and last egg to divide is increased or decreased by the same factor as the time for cleavage. It is obvious that sampling and counting errors have least effect where the relative spread of the distribution curves is smallest. Such errors, however, are slight in comparison with the variation that occurs with eggs from different individuals. The magnitude of those errors may be obtained by comparing the time for 50 per cent cleavage of two batches of eggs from the same female run at the same time and under the same conditions. For the eggs of *Ciona*, *Dendraster*, and *Urechis* such a comparison gave differences of less than ½ per cent. *Strongylocentrotus* and *Lytechinus* gave differences of less than 1 per cent. The variation that is obtained with eggs of different individuals is contained in the data presented in the following sections.

In order to compare the effects of temperature on the different stages of development it is evidently best to use as far as possible the data for a single batch of eggs. Since, however, it was not always feasible to obtain all of the stages from a single batch of eggs, the results with different batches are also presented.

Ciona intestinalis

The data that have been collected on this form concern mainly the cleavage stages. The later stages require rather detailed investigation to insure comparison of identical stages for embryos raised at different temperatures. Furthermore, this could be done more easily with the sand dollar and the sea urchin.

The data for hatching time was in many cases obtained. As shown above, however, hatching time may not bear a very definite relation to the time of attainment of the various morphological stages. In *Ciona* hatching occurs after the tadpole is fully formed, and it evidently

results from the liberation of an enzyme (Berrill, 1929). According to the data presented above (p. 61), hatching occurs earlier in a concentrated than in a dilute culture of embryos, presumably because a higher concentration of the enzyme is present. It is conceivable that even in cultures containing the same concentration of embryos temperature may affect the time of hatching in a manner different from its effect on the time of attainment of the various morphological stages. According to the data presented below, the temperature coefficients obtained for hatching are somewhat different from those for the cleavage stages. On the other hand, the temperature coefficients for the various cleavage stages are very much alike.

The data in Table I give the result of three separate runs, one at 20° and 15°, one at 25° and 15°, and one at 22° and 12°. In the first two columns of each group the time at which 50 per cent of the eggs have

TABLE I

Ciona; times of attainment of various stages for three batches of eggs, each run at two different temperatures; the time for the cleavage stages is given in minutes, that for hatching, in hours; the coefficients, Q_5 and Q_{10} are the ratios of the time at the lower temperature to the time at the higher temperature.

	20.0°	15.0°	Q_5	25.0°	15.0°	Q_{10}	22.0°	12.0°	Q_{10}
2-cell	47.2	77.0	1.63	34.9	77.0	2.21	41.9	118.5	2.83
4-cell	69.0	114.0	1.65	50.5	114.5	2.27	64.0	178.0	2.78
8-cell	91.7	149.0	1.63	66.1	150.5	2.28	78.5	224.5	2.81
16-cell	116.0	195.0	1.68	86.0	194.5	2.26			
Hatching	14.7	25.4	1.73	10.2	25.5	2.50	11.5		

reached the indicated stage is given. In the third column are the ratios (Q_5 or Q_{10}) of these values for the lower and higher temperature. In Table II a summary of these ratios for a number of determinations is given. The data are presented for each pair of temperatures separately since, as pointed out in the previous section, each batch of eggs was run at only two different temperatures. The variation in time of development that is obtained with eggs from different individuals may be seen from the following figures for the time of first division in five separate runs at 20.0° C.:

47.2 48.2 47.0 48.3 48.0 Av. = 47.7 a.d. = 0.5

The variation that is obtained at other stages and at other temperatures is of the same relative order of magnitude. The ratios, as shown in Table II, vary less on the whole than do the values for the time of division. In other words, even when the time of development is different in two batches of eggs run at the same two temperatures, the

ratios of the time at the different temperatures for each batch tend to be more nearly alike.

It can be seen from Tables I and II that the temperature coefficients for the rate of attainment of the various cleavage stages are alike. We need not present the deviation measures to demonstrate this, since it

TABLE II

Ciona; temperature coefficients; Q_5 and Q_{10} represent the time at the lower temperature divided by the time at the higher temperature.

	Q_5	Q_{10}	Q_{10}
	15.0°/20.0°	15.0°/25.0°	12.0°/22.0°
2-cell	1.63 1.66 1.64 1.66 1.63	2.21 2.30 2.24 2.25	2.83 2.91 2.85 2.86
	1.63	2.25	2.86
4-cell	1.65 1.65 1.67 1.63	2.27 2.32 2.29 2.27	2.78 2.81 2.83 2.85
	1.65	2.29	2.84
8-cell	1.63 1.64 1.62	2.28 2.32	2.81 2.85
	1.63	2.30	2.83
16-cell	1.68 1.67 1.63	2.26 2.25	
	1.66	2.26	
Hatching	1.73 1.76 1.74	2.50 2.61	
	1.74	2.56	

can be readily seen from Table I that for the same batch of eggs there are no consistent deviations in the coefficients for different stages, and from Table II that for different batches of eggs the range of variation of the average coefficients for the different stages lies within the range of variation of the coefficients obtained for one stage.

The temperature coefficients for hatching, however, are apparently greater than for the cleavage stages. At the lowest temperature employed, namely 12°, no hatching was obtained in four separate experiments, although development appeared to be normal in all cases. In three other runs at this temperature cleavage was interfered with. As a possible interpretation of the effect of temperature on hatching, the following might be suggested. Let us assume that hatching depends upon the attainment of a certain concentration of a particular enzyme and that the effect of temperature on the rate of production of

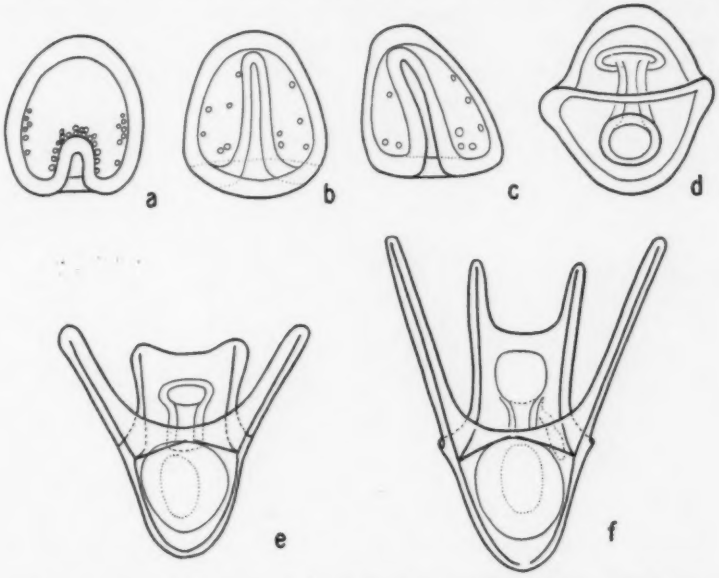

FIG. 1. *Dendraster* embryos. *a*, beginning gastrula; *b*, early prism stage, dorsal view; *c*, early prism stage, side view; *d*, late prism stage, ventral view; *e*, middle pluteus stage; *f*, late pluteus stage.

the enzyme is the same as its effect on the rate of development. The rate at which the enzyme diffuses away from the embryo as well as its rate of production will then determine its concentration in the membrane. Since the physical process of diffusion would have a lower temperature coefficient than the chemical processes involved in the production of the enzyme, the rate of diffusion would not be slowed up as much, when the temperature is lowered, as the rate of production. This would lead to larger values for the temperature coefficient of hatching as compared with that for development. For the failure to

hatch at a low temperature at which development is normal, we might assume in addition that the enzyme must attain a certain minimum concentration in order to be effective. Considerably more information than has yet been obtained would be necessary to test these points.

Dendraster excentricus

In this form temperature coefficients for gastrulation, prism and pluteus stages as well as for cleavage stages were determined. In a few cases hatching-time was also noted. Some of the stages examined are illustrated in Fig. 1. During gastrulation embryos that are 1/8, 1/4, 1/2, 3/4 or completely gastrulated can be readily distinguished. At the completion of gastrulation the sand-dollar embryo has become flattened on one side (the anterior side in consideration of the direction of

TABLE III

Dendraster; same description as Table I

	20.0°	15.0°	Q_5	15.0°	10.0°	Q_5	22.0°	12.0°	Q_{10}
2-cell	55.0	89.5	1.63	90.0	184.0	2.04	46.8	135.0	2.88
4-cell	86.5	138.0	1.60	139.5	281.0	2.01	72.0	202.0	2.81
8-cell	109.0	173.0	1.59	174.0	336.0	1.93	98.0	278.0	2.84
16-cell	126.0	203.0	1.61	—	—	—	—	—	—
Hatching	7.6	11.8	1.55	—	—	—	7.0	17.4	2.49
1/8 gastrulated	12.7	20.9	1.65	20.2	39.8	1.97	10.9	30.4	2.79
1/4 gastrulated	13.2	21.5	1.63	—	—	—	—	—	—
1/2 gastrulated	14.2	22.9	1.61	23.1	46.0	1.99	—	—	—
3/4 gastrulated	15.0	24.1	1.61	24.6	49.2	2.00	12.6	36.0	2.82
Early prism	15.9	26.4	1.66	26.3	53.0	2.01	—	—	—
Late prism	20.8	33.1	1.59	—	—	—	—	—	—
Early pluteus	23.5	38.6	1.64	38.0	77.5	2.04	—	—	—
Middle pluteus	33.1	54.6	1.65	55.0	113.5	2.06	—	—	—
Late pluteus	—	—	—	—	—	—	48.7	142.0	2.92

swimming of the pluteus), and may at that time be designated as the early prism stage (Fig. 1*b* and *c*). Later, that side of the embryo becomes concave and the archenteric pouches appear. We may designate this stage as the late prism (Fig. 1*d*). Following this the aboral (ventral) arms appear and the gut becomes tripartite, forming the early pluteus. The next stage is just before the appearance of the oral (dorsal) arms, and may be called the middle pluteus stage (Fig. 1*e*). Before this stage the anterior border of the oral lobe is convex and as the oral arms form it becomes concave. This change takes place fairly rapidly and so the time at which this stage is reached can be determined with some accuracy. For the late pluteus stage (Fig. 1*f*) the length of the oral arms serves as a simple criterion in identifying embryos that are comparable.

Table III gives the data for single batches of eggs, one run at

20.0° and 15.0°, one at 15.0° and 10.0° and one at 22.0° and 12.0°. The ratios of the time at the lower temperature to that at the higher temperature are given in the third, sixth and ninth columns of the table (Q_5 and Q_{10}). In Table IV the values of these ratios for five pairs of temperatures are given. Each value is, of course, obtained

TABLE IV

Dendraster; same description as Table II

	15.0°/20.0°	10.0°/15.0°	12.0°/22.0°	15.0°/25.0°	8.0°/18.0°
2-cell stage	1.63	2.04	2.88	2.21	3.91
	1.64 1.61	1.95	2.78	2.18	3.85
	1.59 1.61	2.00	2.79	2.25	
	1.63	2.06	2.82		
	1.62	2.01	2.82	2.21	3.88
4, 8 and 16-cell stage	1.60	2.01	2.81	2.26	3.82
	1.62	1.92	2.78 2.75	2.22	
	1.58	1.94	2.80 2.85		
	1.59		2.78		
	1.60	1.96	2.80	2.24	3.82
Gastrulation	1.65	1.97	2.79	2.17	3.83
	1.63 1.66	1.99	2.82	2.22	3.94
	1.61 1.62	2.00	2.79		
	1.61 1.61	1.93	2.76		
	1.58 1.59	2.00			
	1.59 1.60	2.00			
		2.03			
	1.60	1.99	2.79	2.20	3.98
Prism	1.66	2.01	2.79	2.17	3.90
	1.59	2.08			3.82
		2.02			
	1.63	2.04	2.79	2.17	3.86
Pluteus	1.64	2.04	2.85		3.86
	1.65	2.06	2.92		4.01
		2.03	2.76		3.95
		2.00	2.82		
	1.65	2.03	2.84		3.94

from a single batch of eggs run at the two temperatures. In some cases values for a number of stages (as is the case in Table I) were obtained from a single batch of eggs. It can be readily seen from the tables that the temperature coefficients for the various cleavage stages are the same. Furthermore, the coefficients for the later stages fall

TABLE V

Urechis; same description as Table I.

	20.0°	15.0°	Q_5	22.0°	12.0°	Q_{10}
2-cell.....	74.5	120.0	1.61	65.0	180.5	2.78
4-cell.....	104.0	162.0	1.56	88.0	249.0	2.83
8-cell.....	129.0	206.5	1.60	113.5	319.0	2.81
16-cell.....	158.5	254.5	1.61	39.5	385.0	2.76

within the range of values obtained for the cleavage stages. The range of temperatures (8.0° to 25.0°) covered is practically that outside of which normal development can no longer be obtained.

If the figures obtained for *Dendraster* are compared with those for *Ciona* it may be seen that they are very much alike. The average values for all the cleavage stages are given in Table XIII along with those of the other forms investigated. This similarity in the coefficients for *Dendraster* and *Ciona* may best be discussed after considering the other forms.

Urechis caupo

Only the data for the cleavage stages of *Urechis* eggs are presented here, inasmuch as they present the same difficulties as *Ciona* for obtaining reproducible results in the later stages. The results given in Tables V and VI show that for the eggs of this form too the temperature

TABLE VI

Urechis; same description as Table II.

	15.0°/20.0°	10.0°/15.0°	12.0°/22.0°
2-cell	1.61	1.95	2.78
	1.59	2.08	2.76
	1.58	2.15	2.84
	1.60		
	1.64		
	1.63		
	1.61	2.06	2.79
4, 8 and 16-cell	1.56	1.97	2.83
	1.62	2.04	2.79
	1.65	2.11	2.81
	1.60		2.71
	1.63		2.78
	1.59		2.76
	1.61		2.82
	1.61	2.04	2.79

coefficients of the rate of attainment of the various cleavage stages are alike. It may also be readily seen that the values obtained with *Urechis* eggs are quite similar to those obtained with eggs of *Dendraster* and *Ciona* at the same temperatures.

Strongylocentrotus purpuratus

Gastrulation and prism stages as well as cleavage stages of the *Strongylocentrotus* eggs were examined. The extent of the gut cavity

TABLE VII

Strongylocentrotus; same description as Table I.

	20.0°	15.0°	Q_5	20.0°	7.5°	$Q_{12.5}$
2-cell	77.0	105.0	1.36	76.3	227.0	2.98
4-cell	126.0	174.0	1.38	125.0	383.0	3.06
1/8 gastrulated	25.0	32.7	1.31	24.7	73.5	2.98
1/2 gastrulated	28.8	37.0	1.29	28.5	89.0	3.12
3/4 gastrulated	31.4	42.0	1.34	—	—	—
Prism with skeletal rods	49.0	59.0	1.34	48.0	140.0	2.92

TABLE VIII

Strongylocentrotus; same description as Table II.

	Q_5 15.0°/20.0°	$Q_{12.5}$ 7.5°/20.0°
2- and 4-cell	1.36	2.98
	1.30	2.89
	1.31	2.97
	1.38	3.08
	1.30	3.06
	—	—
	1.33	3.00
Gastrulation	1.31	2.98
	1.37	3.04
	1.29	3.12
	1.39	3.09
	1.37	
	—	—
	1.35	3.06

rather than the extent of invaginated cells was used as a criterion for determining the stage of gastrulation. This was done because the primary mesenchyme that covers and extends forward from the gut does not change very markedly in appearance as gastrulation proceeds. Although the results with *Strongylocentrotus* vary more than with the forms previously presented, it may be readily seen from Tables VII and VIII that no consistent differences occur in the

TABLE IX

Lytechinus; same description as Table I.

	15.0°	10.0°	Q_{10}
2-cell	137.0	281.0	2.05
1/8 gastrulated	30.2	63.5	2.08
1/2 gastrulated	35.0	73.0	2.09
Early prism	58.5	116.5	1.99
Late prism	73.0	151.0	2.07

temperature coefficients of the cleavage and the later stages. The average values of the temperature coefficients for *Strongylocentrotus* differ considerably from those for *Ciona*, *Dendraster* and *Urechis* at the same temperatures, as may be seen in Table XIII.

TABLE X

Lytechinus; same description as Table II.

	Q_{10} 10.0°/15.0°
Cleavage	2.05
	1.91
	1.87
	—
	1.94
Gastrulation	2.08
	2.04
	2.09
	—
	2.07
Prism	1.99
	2.10
	2.07
	—
	2.05

Lytechinus anamesus

The time of attainment of the two-cell stage, gastrulation and prism stages was determined for the eggs of this sea urchin at only one pair of temperatures. The results are presented in Tables IX and X. The data are less extensive than for the other forms; nevertheless it can be seen that the temperature coefficients for the later stages are very much like those of the two-cell stage. A comparison of the average value with those of the other forms is given in Table XIII.

Number of Cells in Sea-urchin Gastrulæ Raised at Different Temperatures

From the similarity of the temperature coefficients of the gastrula and the early cleavage stages, it is to be expected that the number of cells present in gastrulæ raised at different temperatures should be the same, provided, of course, that the later cleavage stages have the same temperature coefficient as the earlier ones. Since the data in the early literature (see above) showed different numbers of cells present in embryos raised at different temperatures, this point was re-investigated.

Two methods were used in estimating the cell number, that of Marcus (1906) and that of Koehler (1912). Marcus' method consisted in counting the number of nuclei (n) in an optical section at a great circle. Then the total number of cells (N) is given by

$$N\left(\frac{2\pi r}{n}\right)^2 = 4\pi r^2,$$

where r is the radius of the embryo. It is assumed that the surface outline of each cell is a square. This equation reduces to

$$N = \frac{n^2}{\pi}$$

and it is unnecessary to measure the radius as Marcus did. Koehler counted the number of nuclei in a small area of the surface. The number of nuclei per unit area (n/s^2) multiplied by the surface area of the embryo gives the total number of cells

$$N = 4\pi r^2 \cdot \frac{n}{s^2},$$

where the radius, r, is measured to the middle of the wall. It would be better, however, to measure the radius to a point one-third the distance between the inner and outer surfaces of the wall, since the centers of the nuclei correspond to about that position.

For embryos that have gastrulated corrections must, of course, be made for the invaginated cells, if the total cell number is desired. The measurements presented here were made on embryos that had just begun gastrulation. The number of mesenchyme cells could be estimated roughly and the small invagination could be considered to occupy the same area as if the cells were still on the spherical surface. The surface area was calculated from the formula for an oblate spheroid. Neither of the two methods gave very accurate results. Koehler claims that his method is better since one is not troubled with having the optical section coincide with the greatest circle and with higher and

TABLE XI

Strongylocentrotus; number of cells in beginning gastrulæ.

A		*B*	
20.0°	7.5°	20.0°	7.5°
680	680	776	688
769	799	780	815
623	680	705	863
651	738	690	752
799	708	802	885
799	644	801	829
769	597	854	780
680	608	806	809
721	682	777	803

lower nuclei that might or might not be in the great circle. But in Koehler's method the unit square of surface in which the nuclei are counted is actually a spherical surface and if a large area is taken, then too many nuclei are counted, whereas if a small area is taken the error due to the curvature is negligible but the counting becomes more difficult because a considerable fraction of the nuclei are cut by the boundaries of the area.

In Table XI the cell numbers are given for eight beginning gastrulæ raised at 20.0° and eight of the same batch raised at 7.5° C. The counts were made on slides of preserved embryos, only those in side view being chosen. The first two columns (*A*) were obtained from counts of the nuclei in optical section. The second two columns (*B*) are from counts of nuclei in a unit area of surface. Figures 2*a* and *b* illustrate the nuclei in optical section and Figs. 2*a'* and *b'* the surface nuclei of a 20.0° and a 7.5° embryo. These two embryos happened to show the same number of nuclei (45) in optical section and gave a total (including 35 mesenchyme cells) of 680 cells. The cell numbers from the counts of the surface nuclei are 776 and 688 respectively. The other values presented in the table vary considerably, yet there are no consistent differences between the warm and cold series.

DISCUSSION

Numerous methods have been employed by biologists to express the relation between temperature and the rate of biological processes. An extensive review is given by Bělehrádek (1935). Although we are not especially concerned here with establishing any particular relation,

it is of some interest to examine how well two of the commonly employed formulæ fit our data. We shall consider first the Arrhenius equation which has been extensively applied by Crozier and his collaborators (1924–) to various biological processes including the rate of development. If the logarithm of the velocity of the particular process is plotted against the reciprocal of the absolute temperature, then, according to the Arrhenius equation, a straight line should be expected. In the graph of Fig. 3 the data for the first cleavage of

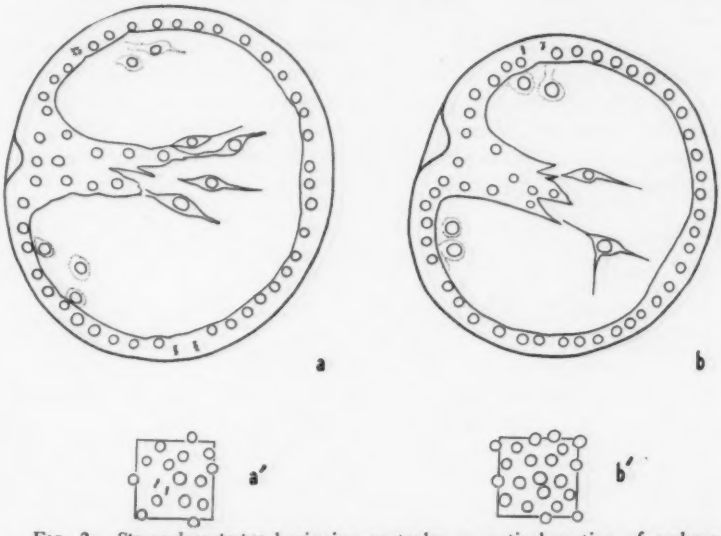

FIG. 2. *Strongylocentrotus* beginning gastrulæ: *a*, optical section of embryo raised at 20.0° C.; *a′*, nuclei in unit area of surface of same embryo; *b*, optical section of embryo raised at 7.5° C.; *b′*, nuclei in unit area of surface of same embryo.

Ciona, *Dendraster* and *Urechis* are so plotted. Only the values for the first division were used since these were the most reliable ones obtained. The figures from which the graph was obtained are given in Table XII. These figures do not represent averages of all the first cleavage values but were taken from cases where the cleavage time obtained at one temperature was practically identical in two separate runs in which the temperature of the second bath had been changed. This was done in order to cut down the variability that would be obtained with different batches of eggs and, of course, because only two temperature baths were available at one time. The graphs of Fig. 3 are certainly not straight lines. Small portions of these graphs might be considered as such. But in order to conclude that the

TABLE XII

Time for first cleavage.

	Ciona	Dendraster	Urechis
8.0° C.		248.0	
10.0		184.0	262.0
12.0	117.9	135.0	183.5
15.0	77.0	89.5	120.0
18.0	56.5	63.5	89.5
20.0	47.7	54.75	74.5
22.0	42.2	46.75	64.5
25.0	35.1	40.5	

Arrhenius equation applies we would need to make the now prevalent but as yet unproved assumption of different limiting reactions of a catenary series at different temperature intervals. If such an assumption were to be made, it would be desirable to have considerably more points on the graph.

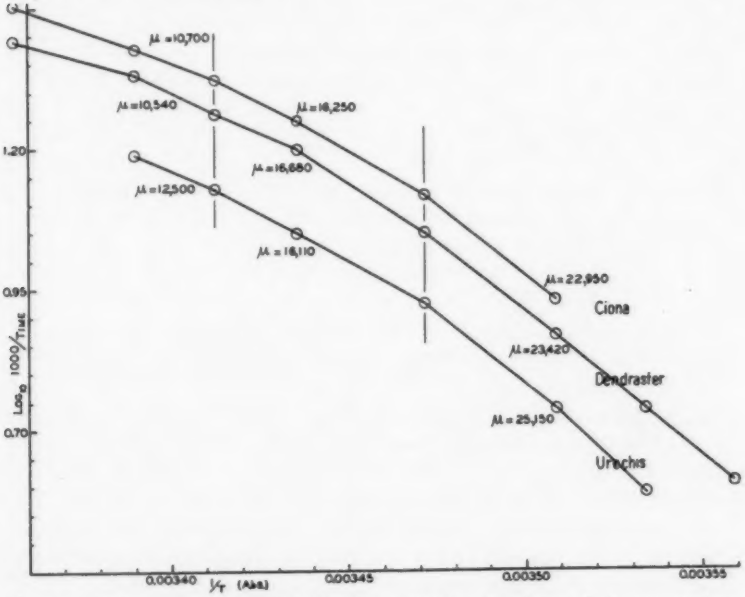

FIG. 3. First cleavage data of Table XII plotted as logarithm of the rate against the absolute temperature.

There is a rather simple relation between temperature and the rates of biological processes that was first applied to developing eggs by Krogh (1914). This relation is simply that the rate is directly pro-

portional to the temperature. Support for this view has been obtained by a number of investigators, and recently Ephrussi (1933) has shown that the effect of temperature on the rate of cleavage of sea-urchin eggs is fairly well expressed by it. In Fig. 4 the data of Table XII are plotted as the reciprocal of the time for first cleavage against the temperature. The points fall fairly well on a straight line for most of the temperature range, as they should if the rate is directly proportional to the temperature. At the lower temperatures deviations occur in the graphs for *Dendraster* and *Urechis*. The graph for *Ciona*, the data for which are more reliable than in the case of the other forms, is a very good straight line.

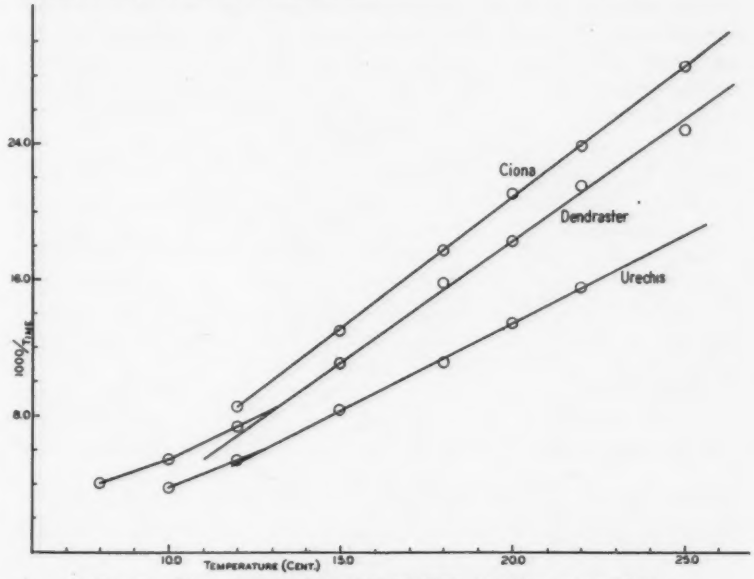

FIG. 4. First cleavage data of Table XII plotted as rate against centigrade temperature.

The significance of this linear relation between the rate of a biological process and temperature is quite obscure. Bělehrádek employs a modified equation which may be written

$$v = a\,(t - \alpha)^b$$

in which v is the velocity, t the temperature and a, α and b constants. When the exponent $b = 1$, this becomes the linear relation. It is assumed that the temperature coefficients give a measure of the relative viscosity although just how the constants of the formula are

related to viscosity is not clear. Support of this view is given by Bělehrádek and Mládek (1934), who found higher values for the temperature coefficients of respiration of more viscous meal-worm larvæ than of less viscous ones. Bodine and Thompson (1935) showed, however, that this is not necessarily the case, inasmuch as dehydrated grasshopper embryos gave the same values for the temperature coefficient of respiration as normal embryos.

If the temperature coefficients for the five forms investigated are compared, it is seen that four of them, *Ciona*, *Dendraster*, *Urechis* and *Lytechinus*, are very similar, whereas *Strongylocentrotus* gives values that are quite different. In Table XIII the mean values for the cleavage stages are presented. An examination of the values for the temperatures 15° and 20° (first column of table) shows that the coefficient for *Strongylocentrotus* is much lower than for the others. At the temperatures 7.5° and 20° the figure for *Strongylocentrotus* in

TABLE XIII

Temperature coefficients for cleavage. (Mean values and their standard deviations taken for all the cleavage data.)

	Q_5	Q_{10}	Q_2	Q_{10}	$Q_{12.5}$
	15°/20°	15°/25°	10°/15°	12°/22°	7.5°/20°
Ciona	1.65±0.018	2.27±0.033	———	2.84±0.035	———
Dendraster	1.61±0.02	2.22±0.032	1.99±0.053	2.80±0.038	4.7*
Urechis	1.61±0.026	———	2.05±0.079	2.79±0.029	———
Lytechinus	———	———	1.94	———	———
Strongylocentrotus	1.33±0.037	———	———	———	3.00±0.076

* Calculated value.

this table is 3.0, whereas the value for *Dendraster* in the temperature interval 8° and 18° is 3.9 (Table IV) and the extrapolated value for 7.5° and 20° is 4.7.

In view of the striking difference shown by *Strongylocentrotus*, it is hardly worthwhile at present to try to account for the similarity shown by the others. The closeness of taxonomic relationship evidently has no bearing on the results, inasmuch as the two other echinoderms, *Dendraster* and *Lytechinus*, differ from *Strongylocentrotus*, whereas they are like *Ciona* and *Urechis*. It also appears that the eggs of one species may have different temperature coefficients, as Ephrussi (1933) has shown for the results of Horstadius (1925) on the rate of cleavage of *Paracentrotus lividus* eggs in summer and in winter. This particular case is taken by Ephrussi to illustrate the rule of Skrabal (1916) that fast reactions have lower temperature coefficients

than slow reactions. Zawadowsky and Sidorov (1928) present values for the cleavage of sea urchins (three species), frog and *Ascaris* eggs that are in agreement with that rule. The data presented in this paper, however, do not fit into that scheme even in the case of the three echinoderms alone. For example, *Strongylocentrotus* has a slower rate of cleavage than the other two echinoderms and also a lower temperature coefficient.

It is the relation between the temperature coefficients for cleavage and for organogenesis that is of particular interest here. That the various early cleavage stages have the same temperature coefficients is perhaps to be expected since the early cleavage stages represent the same sort of process. This statement must be qualified, however, for development up to the first cleavage since in addition to the kind of events occurring in the later cleavages there is also fertilization, fusion of pronuclei, extrusion of polar bodies, etc. to be considered. The temperature coefficients for the various phases of the first cleavage have been investigated by Ephrussi (1927) and for fertilization alone by Chase (1935). Different values are found by Ephrussi for various stages from fertilization to first division in *Paracentrotus*, but this would not prevent the overall values for the various cleavage stages (including the first) from being the same. Fry (1936) finds, on the other hand, the same temperature coefficient for the mitotic phases as for cleavage in *Arbacia*. The kinds of changes that occur in the later stages of differentiation (i.e. from gastrulation on) are manifestly different from the cleavage stages. The interpretation that may be made of the identity of temperature coefficients throughout development depends, of course, on the particular meaning assigned to temperature coefficients in general. But this involves some controversial questions that are beyond the scope of this paper.

Our results show that we cannot within the range of temperatures used dissociate cleavage and organogenesis by means of temperature. It would be of interest to know whether other agents or extremes of temperature might effect such a separation so that the rates may be affected differently or that a process such as gastrulation may be made to proceed without the accompanying cell division.

Summary

The effect of temperature on the rate of development of eggs of *Ciona intestinalis*, *Dendraster excentricus*, *Urechis caupo*, *Strongylocentrotus purpuratus* and *Lytechinus anamesus* has been investigated. It is found that not only do the various early cleavage stages have the same temperature coefficient, but the values for the later stages of differentiation are the same as for cleavage.

In *Ciona*, the coefficient for hatching time is different from that for cleavage. An interpretation of this based on the production and diffusion of a hatching enzyme is suggested.

Counts of the number of cells of gastrulæ of *Strongylocentrotus* show no significant differences in embryos raised at different temperatures.

The data are fairly well expressed by the assumption of a linear relation between the rate of development and temperature.

The values of the coefficients for all the forms investigated, with the exception of *Strongylocentrotus*, are very closely alike, although the rates of development are quite different. *Strongylocentrotus* eggs, which develop more slowly than *Dendraster* or *Lytechinus*, give lower values of the temperature coefficient.

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ON THE ENERGETICS OF DIFFERENTIATION. IV

Comparison of the Rates of Oxygen Consumption and of Development at Different Temperatures of Eggs of Some Marine Animals

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The results of these experiments show principally that, for the eggs of certain marine animals, the total oxygen consumed in reaching the same stage of development at different temperatures is the same.

Theoretical Part

It has been assumed (Tyler, 1933, 1935) that energy is required for the differentiation as well as the maintenance and growth processes of development and that this energy is supplied by reactions involving oxygen.

The terms maintenance, growth and differentiation have been used in various ways by different investigators. By maintenance I mean the processes by which the egg or embryo is kept alive in a "resting condition." An unfertilized egg illustrates this. Also a fertilized egg in which development has been reversibly stopped would illustrate maintenance. But it cannot be assumed that the maintenance energy requirement of a developing egg is equivalent to the metabolism of an unfertilized or of a fertilized egg in which development has been stopped, for the maintenance requirement of a fertilized egg is probably different from that of an unfertilized egg; it very likely changes throughout development and under different conditions. In other words, it takes different amounts of energy to keep different kinds of cells alive under the same conditions, and the same kind of cell alive under different conditions.

By growth we mean the processes by which food material is converted into protoplasm. This may not be the most convenient way in which to define this term in the case of the early development of a marine egg. It is, however, more consistent with the generally employed definitions of growth. The difficulties arise in distinguishing between the food materials and the protoplasm of an egg. We recognize, how-

ever, certain constituents such as the yolk, fat, and some pigments as essentially non-protoplasmic materials. In the case of most small eggs these materials are distributed throughout the cytoplasm. In the case of large eggs, such as those of birds, a region which is preponderantly protoplasmic may be distinguished from one which is chiefly yolk. The embryo arises in the protoplasmic area and evidently contains a negligible amount of food material. Thus in studies on the growth of bird or fish embryos it is essentially the conversion of food materials into protoplasm that is being measured. In the case of unicellular organisms growth studies likewise give a measure of the conversion of food materials of the medium into protoplasm of the cells. For small eggs it has been the custom either to consider growth as not occurring until actual increase in mass by absorption of materials from the outside takes place, or to use the term growth as loosely synonymous with development. It is evident, however, that there is growth in small eggs during the period before actual increase in mass takes place. The difference is that the nutritive material is intracellular.

Distinction should also be made between growth and what we may call storage. The increase in size of the young oöcyte as it is transformed into the ripe egg may be considered as illustrating storage since it is principally food materials that accumulate. It may seem that this manner of regarding growth leads to a paradox; we have cases of no increase in mass in which we do not consider growth to be occurring. But the ordinary definition of growth as increase in mass is meaningless unless the system is specified. Our definition considers the protoplasm to be the growing system. We define growth in this manner in order to be able when necessary to utilize the general results of growth studies on various kinds of living things.

Differentiation may be regarded as the changes in form that occur during development, the so-called morphogenetic and histogenetic changes. Strictly speaking, this is one aspect of what may be called differentiation. The production of the typical chemical substances characteristic of the various tissues must also be included. However, this also fits into our definition of growth. There is no point at present in attempting to classify it specifically with growth or with the changes in form. It rests, of course, with the actual experimental evidence to decide what the exact relationships are.

The relation of cleavage to differentiation has been considered in the previous paper. In the early experiments of Lillie (1906), a sort of differentiation without cleavage has been described. But it is questionable whether in this case typical differentiation processes have taken place. This is not merely a matter of the use of words, for if the kinds

of changes described by Lillie are comparable with those undergone in normal development, such embryos would furnish important material for the analysis of the form changes and histochemical changes. It has, of course, been long known that cleavage and growth may go on without differentiation (e.g., anidian chick embryos, Dareste, 1882, Grodzinski, 1933). But that should not lead us to expect the reverse to be true.

In distinguishing between maintenance, growth and differentiation we do not mean to imply that these are independent processes in development. They are undoubtedly interrelated, but we can effect a separation conceptually and to some extent experimentally. An excellent review of normally occurring and experimentally produced cases of dissociation of the various embryological processes is given by Needham (1933), and justification for the general point of view presented here is contained therein.

Concerning the effect of temperature on the rate of respiration and on the rate of development, the different processes involved should be considered.

If as the temperature is lowered the requirements for these processes are decreased in the same proportion, we should expect the decrease in rate of development to be the same as the decrease in rate of oxygen consumption. This would not tell us much about maintenance, growth and differentiation, but it would mean that the efficiency with which the available energy is utilized remains the same at different temperatures. If, however, the requirements for the various processes changed in different ways with change in temperature we should not expect such a simple relation. Unfortunately we cannot state *a priori* in what manner the various processes should be affected by temperature. But it is possible to get at this experimentally. Thus it should be possible to learn the manner in which maintenance is affected by studying the effect of temperature on the metabolism and length of life of unfertilized eggs. The duration of the fertilizable condition must also be investigated, since strictly speaking we must regard maintenance as not merely the process of keeping the cells alive but also of keeping them in a condition in which they are capable of functioning normally. Such an investigation involves certain difficulties which remain to be overcome before the results can be presented.

This work concerns only the effect of temperature on the rate of development and rate of oxygen consumption. Several such investigations have been made in the past. The results of Loeb and Wasteneys (1911) are the most often cited and generally accepted. They found the temperature coefficient of the rate of development to be larger than

that of the rate of oxygen consumption. At low temperatures, then, the total oxygen consumed in reaching a given stage of development would be greater than at higher temperatures. Loeb and Wasteneys worked with sea-urchin eggs and considered only the early cleavage stages. But as we have seen in the previous papers, the temperature coefficients for the cleavage and the later stages are the same. They determined their temperature coefficients for respiration by running a batch of eggs for some time at one temperature and then changing the temperature on the same batch of eggs. Thus, the eggs are not in the same stage of development and, as Ephrussi (1933, p. 115) points out, the results are not strictly comparable, since as development proceeds the rate of oxygen consumption rises.

Ephrussi, working also on sea-urchin eggs, gets different results. Using the time from the two-cell stage to hatching as the criterion for rate of development, he finds that the total oxygen consumption is the same at different temperatures, in the interval from 10° to 23°. Above 23° the total oxygen consumption is greater than at the lower temperatures, but Ephrussi states that it is not certain that development would proceed very far at these higher temperatures. When, however, he takes the interval from fertilization to the two-cell stage, he finds the total oxygen consumption to be the same only in the interval between 16° and 23°. Above 23° the total oxygen consumption is higher and below 16° it is lower than at the other temperatures. He supposes that these differences only occur in the early stages and are obscured in the longer time interval and increasingly greater oxygen consumption up to hatching.

Measurements of the rate of cleavage and rate of oxygen consumption at different temperatures have been made by Fauré-Fremiet (1924) on eggs of *Sabellaria*. From his results it appears that the total oxygen consumption in reaching the same stage of development is different at different temperatures.

The same sort of experiment has been performed on eggs of the frog by Barthélemy and Bonnet (1926), using the bomb calorimeter. They found that between 8° and 21° there was the same expenditure of energy in reaching the stage at which the external gills disappear.

In insects, Krogh (1914) and more recently Crescitelli (1935) have determined the rate of pupal development and of respiration at different temperatures. They obtained conflicting results. Krogh used pupæ of the beetle, *Tenebrio*, and found that in the interval of 20° to 33° the total CO_2 produced during pupal life was the same at different temperatures. Crescitelli used pupæ of the moth, *Galleria*, and found in the interval of 20° to 40° the total O_2 consumed or CO_2 produced to

be different at different temperatures. He gets a minimum total gas exchange at 30° C. with regular increases to each side of that temperature. Dobzhansky and Poulson (1935) present some data on pupæ of *Drosophila pseudoöbscura* from which it appears that the total oxygen consumption at 25° and 14° is of the same order of magnitude. Similar results are obtained by Brown (1928) with *Ascaris* eggs and by McCoy (1930) with hookworm eggs.

If in the case of the sea-urchin egg the total oxygen consumed at the lower temperatures were much greater than at the higher temperatures, as the results of Loeb and Wasteneys show, we should expect a rather interesting result. The sea-urchin embryo dies within a limited time after entering the pluteus stage unless food is supplied. If, then, we allow sea-urchin eggs to develop at different temperatures on their own initial food supply, the retardation in time of death will not be the same as the retardation in rate of development but will be the same as the retardation in rate of oxygen consumption, since that measures the rate at which the original food supply is being depleted. For example, if we take Loeb and Wasteneys' figures of 7.3 and 2.0 for the coefficients of rate of cleavage and rate of oxygen consumption for 7°–17°, and assuming (as they do) that the values for cleavage hold throughout development, then development will be slowed up 7.3 times at 7° as compared with 17° but the length of life will be prolonged only 2.0 times. Thus, even allowing for the short time in which the pluteus remains alive without undergoing any developmental changes, we should not expect the embryos raised at 7° to have developed as far at the time of death as those at 17°. In fact, it appears from the figures that the embryos raised at 7° would be expected to die in the late prism stage. Thus below a certain temperature embryos may develop normally but would die before being able to feed, since in the prism stage the mouth is not open. We have investigated this particular point and have found no such precocious dying at the lower temperatures. As is evident in the previous paper, embryos raised at the lower temperatures develop as far as those raised at the higher temperatures. While this sort of evidence shows that enormous differences in the temperature coefficients of development and respiration do not occur with our material, it might well be that smaller differences exist. Our experimental evidence, however, gives no significant differences.

Experimental Part

Material and Methods

The eggs of four marine animals were used; namely, the sand-dollar, *Dendraster excentricus,* the sea-urchin, *Strongylocentrotus purpuratus,* the ascidian, *Ciona intestinalis,* and the gephyrean worm, *Urechis caupo.*

The method of determining the rate of development at different temperatures is given in the previous paper. One or more determinations of the temperature coefficient of the rate of development were made on each set of eggs used for the respiration experiments. For this purpose eggs from the same fertilized lot that were used for oxygen consumption measurements were placed in the temperature baths at the same time as the manometers. These egg suspensions were of about the same concentration as the suspensions in the manometer vessels and were subjected to the same shaking. The eggs in the manometer vessels, used for the respiration measurements, were also examined at the end of each run to check their condition as well as the stage of development.

FIG. 1. Type of manometer vessel used in these experiments.

The respiration was measured by means of the Warburg manometer. The vessels used are of the type illustrated in Fig. 1. They are quite small, the calibration volumes ranging from 2.8 to 3.0 cc., which makes them particularly suitable in cases in which only small quantities of material can be obtained. The manometer capillaries were also small (about 0.6 mm. diameter) and the T connection for the arm that hangs into the bath was made much smaller than usual, thus reducing the gas volume outside of the bath. These vessels have been checked against the standard conical type. The amount of fluid with which the vessels can be filled depends upon the rate of shaking. As much as 1.5 cc. of fluid can be used with moderate shaking (about twenty round-trip shakes per minute). In most of the experiments, however, the vessels were filled with 1.1 cc. of fluid, which gives vessel constants for oxygen at 20° ranging around 0.18. As has often been pointed out (see especially Whitaker, 1933), the rate of shaking is of considerable importance when measurements on marine eggs are being made, for one is concerned not only with insuring adequate diffusion of oxygen but also with avoidance of injury to the material. We have determined the optimum shaking for our material and find slow rates of 40 to 70 round-trip shakes per minute at 3.5 cm. amplitude to be satisfactory for dilute egg suspensions (up to 0.5 mg. of nitrogen in 1.1 cc., which for *Strongylocentrotus* corresponds to a volume concentration of 1 : 100). For more concentrated suspensions stronger shaking is necessary, not only because of the greater oxygen consumption, but also because at

the same rate of shaking the contents are less disturbed when a concentrated egg suspension is employed. For suspensions giving 2.5 mgm. nitrogen in 1.1 cc. the shaking was 70 to 100 round-trips at 4.5 cm. amplitude.

The quantity of eggs employed was determined after each run by measuring the nitrogen content by a micro-Kjeldahl method. Since this determination could be made to 0.01 mgm. N, the error involved when the quantity of eggs corresponds to 1.0 mgm. N is less than 1 per cent. In some instances the actual number of eggs used was determined by counting.

With the higher ratio of fluid to gas volumes obtained in our vessels the solubility coefficient of O_2 (Bunsen's coefficient, α) is of slightly more importance than in the usual type of vessel. Values calculated from the formula of Fox (1909) range from 0.024 at 25° C. to 0.033 at 7.5° C. This term therefore contributes only about 2 per cent to the vessel constant and an error of as much as 10 per cent in the values of α would mean an error of 0.2 per cent in the vessel constant. It is evident then, especially since we are interested in the relative values of oxygen consumption, that errors from this source are negligible.

The manometer readings were made to the nearest 0.5 mm. To determine the pressure changes to which the oxygen consumption data of the following tables correspond it is simply necessary to multiply by the mgm. N of the egg suspension in the vessel and divide by the vessel constant. For example, in Table I, X_{O_2}/mgm. N for the first 20° vessel is 6.7 cu. mm. O_2 in one hour. Multiplying by the mgm. N and dividing by the vessel constant gives 11.5 mm. as the pressure change. Since the reading is made to 0.5 mm. the error is less than 2.5 per cent. This, however, is one of the smallest values presented in the table. If we take the figure 128.8 cu. mm. O_2 from Table II for 5 hours respiration at 20°, this corresponds to a pressure change of 357.5 mm. and the reading error is less than 0.1 per cent.

The measurements were practically all made in duplicate. For the same batch of eggs two vessels were run simultaneously at one temperature and two at a different temperature. Different batches of eggs may vary somewhat in their absolute rates of respiration as well as in their rates of development. To avoid this source of error it is preferable to compare only the results on the same batch of eggs.

Strongylocentrotus

In Table I the results of three experiments with eggs of *Strongylocentrotus* in the early cleavage stages are given. The first column of the table gives the temperature at which the measurements are made;

the second column shows the nitrogen content of the eggs used in each vessel; the third, the age of the eggs as the time from fertilization at 20° C. of the first reading of the manometer; the fourth and fifth give the length of time (from the first reading) during which the oxygen consumption is measured and the corresponding oxygen consumption in cu. mm. O_2 per milligram of egg nitrogen; the sixth and seventh columns again give the respiration time (also from the first reading but continued over a longer time) and the corresponding oxygen consumption.

In the first experiment the Q_5 is 1.36. This was determined from the time between the first and second cleavage for eggs of the same fertilized lot that were placed in the 20° and 15° baths at the same time

TABLE I

Strongylocentrotus; oxygen consumption at different temperatures; early cleavage stages.

Temp.	mgm. N	Time of first reading	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$
° C.		*minutes*	*hours*		*hours*	
20	0.31	45	1.0	6.7	5.0	40.5
20	0.25	45	1.0	6.8	5.0	41.0
15	0.40	45	1.36	6.2	6.8	39.1
15	0.36	45	1.36	6.6	6.8	38.9
20	1.71	35	1.0	6.4	3.5	24.4
20	1.74	35	1.0	6.3	3.5	24.1
10	1.75	35	2.3	6.5	8.1	23.8
10	1.78	35	2.3	6.1	8.1	23.4
20	2.38	50	1.0	6.4	2.5	17.8
20	2.40	50	1.0	6.3	2.5	17.5
7.5	2.39	50	3.2	6.2	8.0	17.6
7.5	2.38	50	3.2	6.3	8.0	17.7

as the manometers and were also at about the same concentration as the eggs in the manometers. The one-hour interval at 20° therefore corresponds to 1.36 hours at 15° and the five-hour interval at 20° to 6.8 hours at 15°; that is, in those time intervals the same stage of development would be attained at the two temperatures. Comparing then the oxygen consumption for the corresponding time intervals at the two temperatures we see that for both the shorter and longer runs the 20° figures are greater. The differences are, however, within the limits of error. It is evident, too, that for 5 and 6.8 hours the differences are much smaller than for 1 and 1.36 hours, which is to be expected if the Q_5 obtained for the rate of development is reliable, since the errors in the oxygen consumption measurements decrease in the longer run.

In the second and third experiments larger amounts of material were used and the differences in total oxygen consumption are much smaller. The Q_{10} of the rate of cleavage in the second experiment is 2.3 and the $Q_{12.5}$ in the third experiment is 3.2, both determined, as before, from the time between the first and second cleavage. It may readily be seen that the differences in total oxygen consumption at the two temperatures are no greater than the differences between the duplicates. The two values for one temperature may even lie between the two for the other temperature, as in the third experiment (20° and 7.5° for 2.5 and 8.0 hours).

It may be concluded then that in the early cleavage stages and between 20° and 7.5°, the total oxygen consumed in reaching the same stage of development is the same at different temperatures. We may next see whether this holds for the later stages. In the preceding paper it was shown that the later stages of development were affected in the

TABLE II

Strongylocentrotus; oxygen consumption at different temperatures; gastrula stage.

Temp.	mgm. N	Time of first reading	*Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$
°C.		*hours*	*hours*		*hours*	
20	0.51	25	1.0	23.6	5.0	128.8
20	0.37	25	1.0	24.0	5.0	129.1
15	0.42	25	1.31	20.9	6.55	119.5
15	0.34	25	1.31	21.6	6.55	120.7

same manner by temperature as were the cleavage stages. It is possible, however, that the rate of oxygen consumption might be differently affected by temperature in the early cleavage stages and in the later stages. One experiment is presented in Table II and another in Table III showing that this is not the case at least for the temperatures 20° and 15° C. Similar experiments are presented in connection with the other forms investigated.

In Table II the differences between the average total oxygen consumption at the two temperatures appear to be fairly large compared with the small differences between the duplicates. These differences are, however, most likely due to the value of Q_5 being too small. Only one determination of Q_5 was made for this batch of eggs and it was taken for the time from fertilization to gastrulation, which is less accurately determined than cleavage time. In Table III the differences in oxygen consumption at 20° and 15° are quite small. The figures in

this table are arranged differently from those in the other tables and should be compared horizontally. The time interval is given only for the 20° runs. The time interval at 15° is 1.37 times that at 20°. This figure is the Q_5 as determined from the time from the first to second cleavage and from first cleavage to beginning of gastrulation at the two temperatures.

Table III also illustrates the rise in rate of oxygen consumption that occurs as development proceeds. Thus the rate at 21 to 24 hours is three times that at 0 to 3 hours. Of course, even in the first three hours the rate is rising, as may be seen in Table I. It is because of the increasing rate of oxygen consumption that we do not compare the rates at different temperatures, but rather the total oxygen consumed by the

TABLE III

Strongylocentrotus; oxygen consumption at different temperatures; cleavage to gastrulation.

Temp. mgm. N Time of first reading.	20° 0.53 45 min.	20° 0.72 18¾ hrs.	15° 0.61 45 min.	15° 0.86 18¾ hrs.
Time interval at 20° C.	$\frac{X_{O_2}}{\text{mgm. N}}$	$\frac{X_{O_2}}{\text{mgm. N}}$	$\frac{X_{O_2}}{\text{mgm. N}}$	$\frac{X_{O_2}}{\text{mgm. N}}$
hours				
0–3	25.4		24.7	
3–6	34.1		33.8	
6–9	41.8		42.9	
12–15	53.7		55.0	
18–21	65.0	67.2	68.1	68.8
21–24	72.1	74.8	74.9	77.2

eggs in undergoing the same developmental changes at the different temperatures.

A technical point is also illustrated in Table III. It is possible that over long periods of time conditions change in the vessels so that an abnormal respiratory rate is being measured towards the end of a long run. Most of the experiments reported here are of relatively short duration and would not be expected to show such effects. The experiment of Table III shows, however, that reliable data may be obtained throughout a run as long as 24 hours. In this experiment two vessels, one at 20° and one at 15°, were started at 45 minutes after fertilization and run for 24 hours (32.9 hours at 15°). The main batch of eggs was divided between two flasks, one kept at 20° and the other at 15° C. At 18 hours after fertilization (24.7 hours at 15°) embryos from these flasks were washed by gentle centrifugation, and two more respiration vessels, one at 20° and one at 15°, were started. It may be seen from

TABLE IV

Dendraster; oxygen consumption at different temperatures; early cleavage stages.

Temp.	mgm. N	Time of first reading	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$
° C.		*minutes*	*hours*		*hours*	
22	1.28	50	1.0	9.7	3.0	33.5
22	0.82	50	1.0	10.7	3.0	34.6
12	0.92	50	2.8	11.0	8.4	35.9
12	0.82	50	2.8	11.7	8.4	36.6
25	0.69	70	1.0	18.3	3.0	59.1
25	0.53	70	1.0	19.5	3.0	65.8
15	0.62	70	2.2	14.8	6.6	54.9
15	0.78	70	2.2	14.1	6.6	53.0

the table that there are no significant differences in the oxygen consumption for the 18 to 21 and 21 to 24-hour intervals between the two vessels that had been run continuously up to that time and the two that had been freshly started.

Dendraster

It is more difficult with eggs of this form to obtain large quantities in which one hundred per cent are fertilized. In two experiments, how-

TABLE V

Dendraster; oxygen consumption at different temperatures; blastula and gastrula stages.

Temp.	mgm. N	Time of first reading	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$
° C.		*hours*	*hours*		*hours*	
20	0.89	13.0	1.0	34.4	3.0	109.1
20	0.94	13.0	1.0	34.2	3.0	108.1
15	0.76	13.0	1.65	33.5	4.95	105.2
15	0.78	13.0	1.65	34.3	4.95	107.5
15	0.87	12.0	1.0	16.4	3.0	51.0
15	0.98	12.0	1.0	15.9	3.0	49.5
10	0.95	12.0	1.95	16.8	5.85	49.6
10	0.75	12.0	1.95	17.4	5.85	52.0
22	0.21	8.5	1.0	24.4	3.0	75.8
22	0.19	8.5	1.0	25.5	3.0	77.1
12	0.21	8.5	2.75	23.2	8.25	77.6
12	0.20	8.5	2.75	23.7	8.25	78.7
22	0.66	10.0	1.0	27.3	3.0	86.4
22	0.36	10.0	1.0	28.1	3.0	87.0
12	0.55	10.0	2.8	29.4	8.4	92.7
12	0.36	10.0	2.8	31.6	8.4	93.8

ever, fairly large quantities of eggs were obtained in which the fertilization was 95 to 97 per cent. The oxygen consumption data are presented in Table IV, one experiment being at 22° and 12°, the other at 25° and 15°. The Q_{10}'s are 2.8 and 2.2 respectively, determined from the time between first and second cleavage. In the first experiment the oxygen consumption is evidently the same at the two temperatures. In the second experiment the values are higher at 25° than at 15°. The differences appear to be significant, but more data would be needed at these temperatures to establish this point. It should be noted that 25° is just about the upper limit at which normal development is obtained.

In Table V the results of four experiments on blastulæ and gastrulæ are presented, one at 20° and 15°, one at 15° and 10° and two at 22° and 12°. Since only top swimmers are taken, it is not necessary to have 100 per cent fertilization at the start. The time of the first reading (column 3 of the table) represents the age of the embryos at 20° and the corresponding stage of development may be obtained from the data in the preceding paper. The temperature coefficients of the rate of development were determined in these cases from the time between fertilization and the beginning of gastrulation, and the respiration times are, as usual, taken from the values of the temperature coefficients. The oxygen consumption data (X_{O_2}/mgm. N) again show the amount consumed during the same developmental period at the two temperatures of each experiment. As was the case with *Strongylocentrotus,* we find here too no significant differences in the total oxygen consumed at the different temperatures.

An experiment at 20° and 15° covering the entire period from fertilization to gastrulation is presented in Table VI. In this experiment

TABLE VI

Dendraster; oxygen consumption at different temperatures; from one-cell stage to end of gastrulation.

Temp.	No. of eggs	Time of first reading	Respiration time	$\frac{1000\ X_{O_2}}{\text{No. of eggs}}$	Respiration time	$\frac{1000\ X_{O_2}}{\text{No. of eggs}}$
° C.		*minutes*	*hours*		*hours*	
20	1653	40	6.0	2.2	15.0	7.9
20	2791	40	6.0	2.0	15.0	7.5
15	2581	40	9.6	2.0	24.0	7.4

relatively few eggs were used in each vessel and the number was determined at the end of the run by counting them all. There were very few immature eggs in this lot, fertilization being about 98 per cent. In one of the 15° vessels the sea water slopped over into the alkali well. But

TABLE VII

Urechis; oxygen consumption at different temperatures; early cleavage stages.

Temp.	mgm. N	Time of first reading	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$
° C.		*minutes*	*hours*		*hours*	
22	1.19	60	1.0	4.4	4.0	18.6
22	1.40	60	1.0	4.9	4.0	21.2
12	1.55	60	2.7	5.9	10.8	23.0
12	2.26	60	2.7	5.2	10.8	21.1
20	1.03	60	1.0	4.0	4.0	17.3
20	1.36	60	1.0	3.9	4.0	17.3
15	1.66	60	1.62	5.0	6.5	18.2

even without the duplicate run at 15° it may readily be seen that the total oxygen consumed at the two temperatures is the same.

TABLE VIII

Ciona; oxygen consumption at different temperatures; cleavage and later stages.

Temp.	mgm. N	Time of first reading	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$	Respiration time	$\frac{X_{O_2}}{\text{mgm. N}}$
° C.		*minutes*	*hours*		*hours*	
20	0.22	60	3.0	28.2	9.0	119.5
20	0.25	60	3.0	29.3	9.0	120.1
15	0.25	60	5.0	28.9	15.0	116.3
15	0.33	60	5.0	27.3	15.0	113.0

Urechis

Two experiments, one at 22° and 12° and one at 20° and 15°, were performed on eggs of *Urechis*. In both 100 per cent fertilization was obtained, as is usual with eggs of this animal. The results are presented in Table VII. The Q_{10} for the rate of cleavage in the 22°–12° experiment is 2.7 and the Q_5 in the 20°–15° experiment is 1.62. In both experiments the total oxygen consumed at the lower temperature is somewhat higher than at the higher temperature. However, considering the variation between the duplicate vessels, and also the error associated with the temperature coefficient determinations for development (see preceding paper), we cannot regard these differences as significant.

Ciona

Two experiments at 20° and 15° were run with eggs of *Ciona*. In the first (Table VIII) a relatively large number of eggs were employed

and the amount determined as usual from the Kjeldahl nitrogen. In the second (Table IX), fewer eggs were used and the amount determined by counting.

TABLE IX

Ciona; oxygen consumption at different temperatures; cleavage and unhatched tadpole stages.

Temp.	No. of eggs	Time of first reading	Respiration time	$\frac{1000\ X_{O_2}}{\text{No. of eggs}}$	Respiration time	$\frac{1000\ X_{O_2}}{\text{No. of eggs}}$
° C.		*minutes*	*hours*		*hours*	
20	1417	60	3.0	1.9	12.0	13.1
20	1273	60	3.0	2.1	12.0	13.3
15	1052	60	5.0	2.1	20.0	12.9
15	1042	60	5.0	2.0	20.0	12.8

There are some technical difficulties in obtaining large quantities of *Ciona* eggs for respiration measurements. Generally several animals must be used and the eggs must be pooled in order to have comparable lots in each vessel. In removing *Ciona* eggs it is very difficult to be certain that no sperm from the same animal is obtained at the same time. This introduces no serious difficulty when eggs from one individual are used since *Ciona* is a self-sterile hermaphrodite. However, to control the time of fertilization when several animals are used it is necessary to mix all the eggs simultaneously and inseminate at the same time. The material of the first experiment was prepared in this manner from four individuals; that for the second experiment came from one individual.

In both experiments fertilization was 100 per cent, which is the rule for *Ciona* eggs removed from the oviduct. The Q_5's as determined from the time from first to second cleavage were 1.67 in both cases. The duplicate runs in these experiments checked very nicely, the biggest difference being 3 per cent. Since the error associated with the temperature coefficients is also of this order of magnitude, it may readily be seen that the differences in total oxygen consumption for the longer runs at the different temperatures are well within the limits of error. For the shorter readings in both experiments the duplicates at the two temperatures overlapped. We may conclude then that in *Ciona,* too, the total oxygen consumed in reaching the same stage of development is the same at different temperatures.

Discussion

In these experiments it is seen that, within the range employed, the effect of temperature on the rate of development parallels its effect on the rate of oxygen consumption. We know, however, that at temperatures at which development is inhibited respiration goes on at a measurable rate. We might therefore expect striking differences as such temperatures are approached. But certain complications enter when we consider inhibiting temperatures. As the temperature is lowered we first reach a point at which cytoplasmic division is interfered with whereas nuclear division proceeds. This point is reached rather abruptly and not after an infinitesimally slow rate of development is attained, as is evident from the fact that in some eggs of a given lot cytoplasmic division is inhibited whereas in others it goes on at an easily measurable rate. For example, in a batch of sand-dollar eggs raised at 7.5° C., about 80 per cent failed to divide while the rest underwent the first cleavage at about 270 minutes after fertilization. The nuclear activity that proceeds when cytoplasmic division has been inhibited must be regarded as a sort of developmental activity. Should we, however, expect the rate of oxygen consumption to drop to zero at temperatures at which all developmental activity has ceased? This is not necessarily the case if there is any significance to the concept of maintenance. If the inhibition by low temperature is reversible then the blocked eggs may be expected to show a basal metabolism characteristic of that temperature. The magnitude need not be the same as that of the unfertilized egg at the same temperature, since, as was pointed out above, the maintenance metabolism of different kinds of cells probably differs. The recent investigations of Whitaker (1931, 1933) bear on this point. Whitaker found that unfertilized eggs of *Chætopterus* and *Cumingia* respire at a higher rate than do the fertilized eggs. If then it were assumed that at any temperature the maintenance component of the developing egg is equivalent to the metabolism of the unfertilized egg, negative values would be obtained for the requirements of the developmental processes. A similar situation arises in one of the interesting series of experiments of Runnström (1935). He finds that the respiration of unfertilized sea-urchin eggs is much more strongly increased on addition of pyocyanine than that of the developing eggs. The eggs may be fertilized in the pyocyanine solution, but the increase in the rate of respiration is very small compared with that in normal sea water. Addition of HCN depresses the rate of the fertilized eggs in pyocyanine but, strangely enough, in-

creases that of the unfertilized eggs, so that at certain concentrations the latter show a higher respiratory rate.

The identity of the temperature coefficients for the rate of development and the rate of oxygen consumption may be interpreted to mean that the maintenance, growth and differentiation requirements are similarly affected by temperature. It might also mean, although it seems more unlikely, that the proportionate requirements for maintenance and growth change but their sum remains the same fraction of the total at the different temperatures. If maintenance is assumed to be the same sort of process, although different in magnitude, in developing eggs and in unfertilized eggs, then, according to the former interpretation, we should expect the respiration of the unfertilized egg to have the same temperature coefficient as that of the fertilized egg. Measurements have been made of the temperature coefficient of the respiration of unfertilized eggs of the sea-urchin by Rubenstein and Gerard (1934). They find it to be different from that of fertilized eggs. The values for the coefficients of the unfertilized egg are higher than for the fertilized eggs, and the big rise in respiratory rate that occurs upon fertilization at ordinary temperatures disappears at higher temperatures. It would appear then that maintenance in unfertilized and in developing eggs is not the same kind of process. There are, of course, a number of assumptions in this argument. The assumption that the same kinds of processes should show identical temperature coefficients is probably the safest of these. But it is also assumed that the respiration of an unfertilized egg is a measure of its maintenance requirement. This is the way in which basal metabolism studies on adults are regarded. But it is quite conceivable that very little, if any, energy is required for maintenance, and that most of that supplied by the oxidations is quite unnecessary. The other assumption is made because, in the absence of any real information as to the nature of maintenance, it appears to be the simplest. For example, the oxygen consumption might be regarded as representing some sort of destructive process in the unfertilized egg (which, in a certain sense, it is, since the metabolites are being destroyed), but calling it destruction is merely a change in terminology. The real point is whether or not the same kind of process occurs in developing eggs.

One conclusion that has been drawn from the dissimilarity of the temperature coefficients of development and respiration (shown chiefly by Loeb and Wasteneys) is that Child's theory of metabolic gradients cannot hold (Crozier, 1926). But regardless of the particular merits of that theory, it is evident that the experimental facts are inadequate

to justify that objection. The recent experiments of Brachet (1934) appear, on the other hand, to favor the view that the most "active" regions have the highest metabolic rate. He found the respiratory rate of the dorsal lip of the blastopore in the frog to be higher than that of the rest of the egg, and Child (1929) regards this region as a metabolic center. However, to conclude that the inductive properties of the dorsal lip of the blastopore are due to the high metabolism is certainly premature. As Brachet himself points out, injury to an egg or tissue raises the rate of oxygen consumption, but it seems quite certain that simple pricking of the egg would not give induction. We may await with interest experiments on amphibian gastrulæ in which the effect of local application of various reversible dyestuffs affecting the rate of respiration is tested.

Summary

The effect of temperature on the oxygen consumption of the eggs of four marine invertebrates, *Strongylocentrotus purpuratus, Dendraster excentricus, Urechis caupo* and *Ciona intestinalis,* was investigated.

With *Strongylocentrotus* eggs the measurements were made in the early cleavage and gastrula stages as well as continuously up to the gastrula stage and at the temperatures 20°, 15°, 10°, and 7.5° C.

With *Dendraster* eggs the measurements were made in the early cleavage, blastula and gastrula stages as well as continuously throughout those stages and at the temperatures 25°, 22°, 20°, 15° and 12° C.

With *Urechis* eggs the measurements were made in the early cleavage stages and at the temperatures 22°, 20°, 15,° and 12° C.

With *Ciona* eggs the measurements were made in the early cleavage stages and up to hatching and at the temperatures 20° and 15° C.

In all cases, with the exception of one run with *Dendraster,* the total oxygen consumed during the same developmental period at the different temperatures is the same within the limits of error of the measurements. The exceptional *Dendraster* experiment showed a somewhat higher total respiration at 25° as compared with that at 15° C. Omitting this case, which needs to be further investigated, it may be concluded that at least in the range of temperatures investigated no optimum exists at which development is accomplished with a minimum oxygen consumption.

I am indebted to Mr. W. D. Humason for assistance in collecting some of the data presented here and to Professor T. H. Morgan for his valuable suggestions.

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PARTHENOGENETIC MEROGONY OR CLEAVAGE WITHOUT NUCLEI IN ARBACIA PUNCTULATA

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Parts of unfertilized *Arbacia* eggs from which the nucleus has been removed, may be activated by parthenogenetic agents, and they will throw off fertilization membranes, and cleave quite like the fertilized nucleate eggs. This combination of parthenogenesis, in which the male nucleus is lacking, and merogony (development of a fertilized enucleate egg fragment), in which the female nucleus is lacking, may be termed "parthenogenetic merogony." It is of particular interest since the resulting non-nucleate organism lacks chromosomes, genes and therefore the hereditary qualities usually associated with the nucleus.

Material—The Parts or Fractions of Centrifuged Eggs

The material has been obtained by breaking apart the mature unfertilized eggs of *Arbacia punctulata* by centrifugal force and using the non-nucleate parts. The method, as previously described (E. N. Harvey, 1931; E. B. Harvey, 1932) consists in centrifuging the eggs in a sucrose solution of the same tonicity and approximately the same density as the eggs. The eggs remain suspended in this solution during centrifugation and are free to elongate and break apart. The stratification of the centrifuged egg is shown in Plate I, Fig. 1, and eggs in the process of stratification and breaking, as they are observed with the centrifuge microscope, are shown in Photograph 1.

The eggs become dumb-bell shape and then usually break into two slightly unequal parts, a larger *white half* containing oil, clear layer, mitochondria and some yolk, and a smaller *red half* containing yolk and pigment (Figs. 2, 6 and Photographs 2, 4).[1] The nucleus is always in the white half under the oil—this is *invariable* in *Arbacia punctulata*. Both the halves will elongate with further centrifuging (Figs. 3, 7 and Photographs 3, 5) and break into quarters; the white half breaks into a larger *clear quarter* containing oil, clear layer and nucleus and practically

[1] The sizes of the individual granules are: oil (spherical droplets) 0.6–1.0 μ; mitochondria (fine granules) 0.6–1.0 μ; yolk (irregular or polyhedral granules) 0.7–1.1μ; pigment (spherical granules) 1.1–1.6 μ. The relative amount of formed bodies as ascertained by E. N. Harvey (1932) is: oil 1 per cent; mitochondria 4.8 per cent; yolk 27.2 per cent; pigment 5.5 per cent.

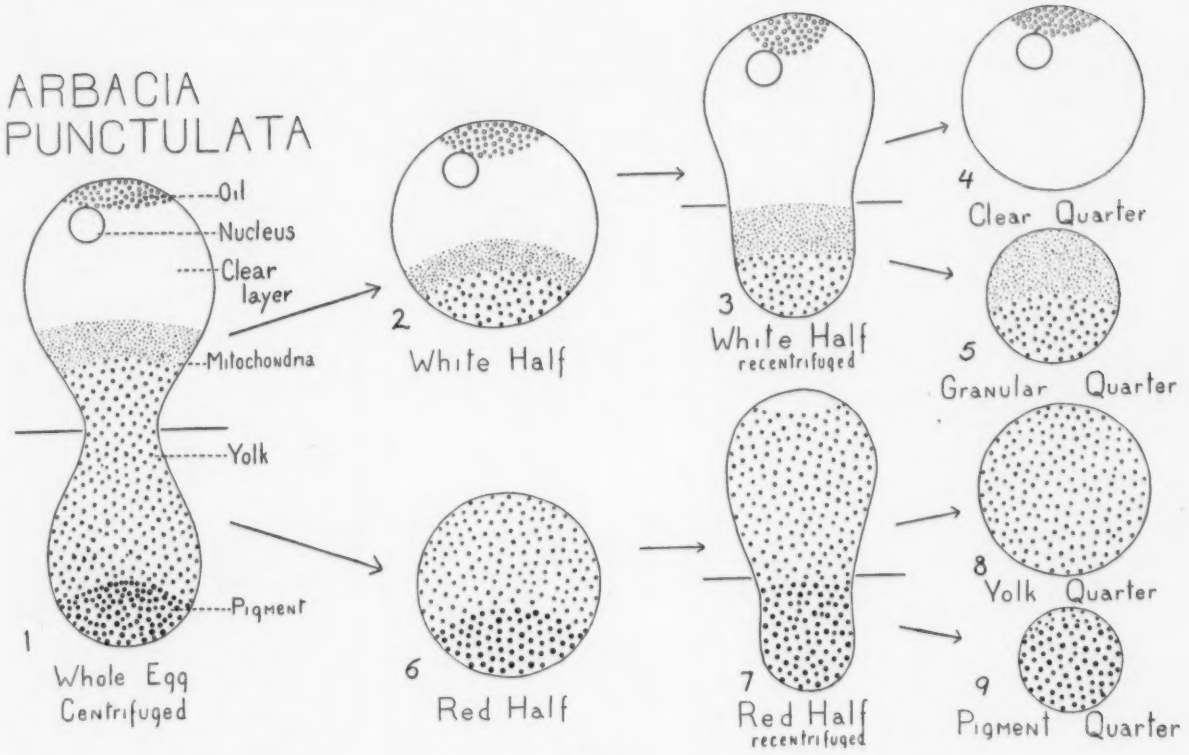


PLATE I

The unfertilized egg of *Arbacia punctulata*, stratified by centrifugal force (about 3 minutes at 10,000 × g.), and the halves and quarters into which it breaks. The drawings are from camera lucida sketches and photographs, made as accurately as possible to scale. Magnified 500 ×.

The clear area in Fig. 7 at the centripetal pole is due to further packing of the granules with longer centrifuging.

no visible granules, and a smaller *granular quarter* containing all the mitochondria and some yolk granules (Figs. 4 and 5 and Photograph 3). The nucleus is invariably in the clear quarter. The red half egg breaks into a larger *yolk quarter* containing only yolk granules, and a very small *pigment quarter* containing all the pigment and a few yolk granules (Figs. 8, 9 and Photographs 1, extreme right, and 5). The halves and quarters are of a very definite and characteristic size and remarkably uniform in any particular batch of eggs (i.e. eggs from one female). The sizes of the halves and quarters (which I will call "fractions") of typical batches are given in Table I; the table is based on the measurements of many eggs from many typical batches and may be considered as a standard.[2] The four parts or fractions which lack a nucleus are marked with asterisks; they are (1) granular quarter, (2) red half, (3) yolk quarter, (4) pigment quarter. There is no possibility of any of these fractions containing any portion of the female nucleus, since in *Arbacia* this is always thrown intact under the oil cap.

TABLE I

Size of the *Arbacia* egg and its fractions obtained by centrifugal force.

Parts	Diameter	Volume	Approximate proportion of whole egg
	μ	μ^3	*per cent*
Whole egg	74	212,000	
White half	62	125,000	60
Clear quarter	56	92,000	40
* Granular quarter	40	33,500	20
* Red half	56	92,000	40
* Yolk quarter	52	73,600	33
* Pigment quarter	32	17,160	7
(Nucleus	11.5	796	0.4)

* Non-nucleate.

Though the great majority of batches of eggs conform quite closely to the sizes given in Table I, there are some consistent variations. A few batches occur each year in which there is a great disparity in the size of the halves, the red halves being very small (Photograph 7); this occurs uniformly throughout the batch irrespective of the speed of centrifuging. Then there occur a few batches which break into almost equal parts, through the mitochondrial layer (Photograph 8).[3] Then there occur in the course of the summer's work one or two batches in

[2] These figures for the relative size of the halves agree closely with those of Shapiro (1935) for slightly smaller eggs.

[3] The measurements of Lucké (1932) to determine the distribution of osmotically inactive material, include two sets (*B* and *D*) of this unusual type.

which the eggs of one batch break at two levels, approximately half of the eggs break into a very small red half and large white half, and the others into a larger red half and a smaller white half (Photograph 9); this gives white halves (and of course the corresponding red halves) of two distinct sizes (Photograph 10). These variations from the standard type of breaking must be due to a difference in the physical structure of the egg, probably viscosity. Such would seem to be the case since the stratification varies with the position of the break; the mitochondrial layer is much better formed when the two halves are nearly equal (*cf.* Photographs 7 and 8). Apart, however, from these infrequent and consistent variations, the material is quite uniform, and there can be no question as to the structure of the various fractions, especially as to whether they are nucleate or not.

Method

For the experimental work, the eggs are centrifuged in tubes a little larger than hæmatocrit tubes, of about 0.7 cc. capacity. The eggs in sea water are placed above a layer of 0.95 molal sucrose solution in the proportion of about $\frac{1}{3}$ sea water to $\frac{2}{3}$ sucrose solution; this is isosmotic and isopycnotic with the eggs. After centrifuging for 3 to 4 minutes at about 10,000 $\times$ gravity, there are usually three distinct layers in the tubes, a layer of white halves above, a pinkish layer of elongate but as yet unbroken whole eggs a little below, and a layer of red halves at the bottom of the tube (Photograph 6, right). With further centri-

Explanation of Photographs 1–6

Centrifuged egg and egg fractions of *Arbacia punctulata*

1. Unfertilized *Arbacia* eggs stratifying and pulling apart as observed in the centrifuge microscope. Photographed while rotating at about 10,000 $\times$ gravity. At the bottom of the slide are the red non-nucleate halves. At the right is one of the red halves pulling apart into quarters; at the extreme right, the yolk quarter above and the pigment quarter below.

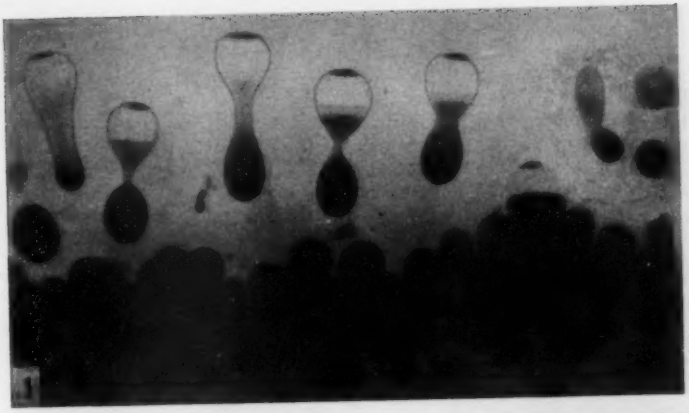
2. White halves showing oil cap (black), clear layer, and granules (mitochondria and yolk). The nucleus invariably lies under the oil cap.

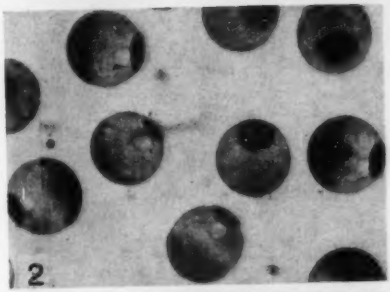
3. White halves (at right and left) elongated by greater centrifugal force, and their two quarters, clear quarter (below) with nucleus under the oil cap, and granular quarter (above).

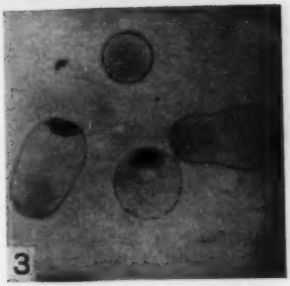
4. Red halves, non-nucleate, containing only yolk and pigment (black).

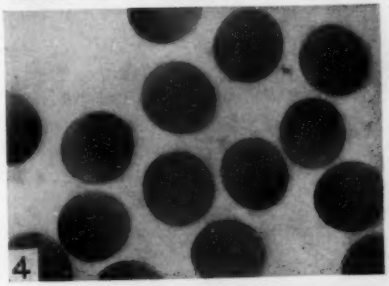
5. Red halves elongated by greater centrifugal force, separating into their two quarters, yolk quarter (larger and lighter) and pigment quarter (smaller and darker).

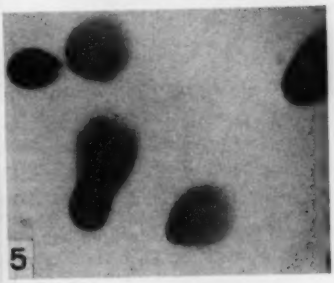
6. Centrifuge tubes (placed upright immediately after removal from the centrifuge), showing masses of eggs and egg fractions in distinct layers. Right tube, three layers: (above) white halves; (center) elongate but as yet unbroken whole eggs; (bottom) red halves. Left tube, five layers: (1, at top) white halves; (2) unbroken whole eggs; (3) yolk quarters; (4) red halves; (5, at bottom) pigment quarters.

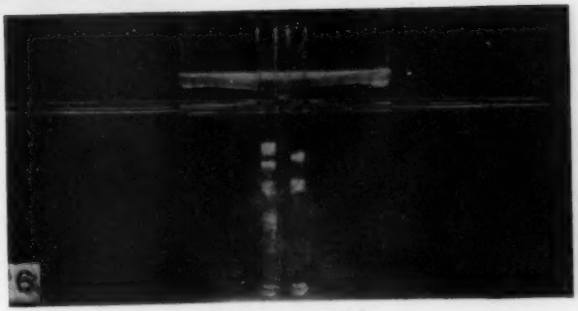




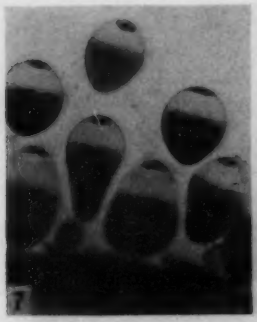


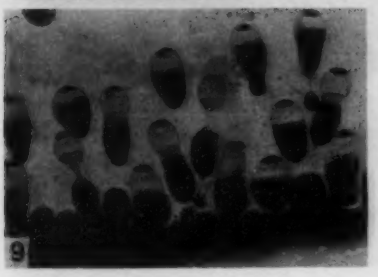


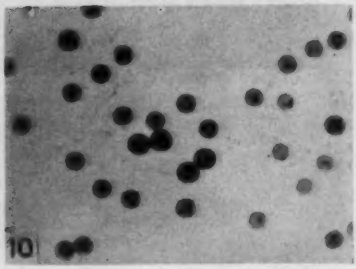


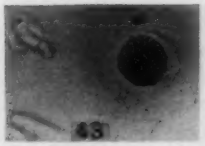


PHOTOGRAPHS 1–6









PHOTOGRAPHS 7–10

PHOTOGRAPHS 7–10 show unusual breaking of unfertilized *Arbacia* eggs. PHOTOGRAPHS 7–9 were taken while rotating on the centrifuge microscope.

7. One broken egg shows unusual breaking into a very large white half (above) and a very small red half (below). Mitochondrial layer not well formed.

8. Unusual breaking into almost equal halves. Mitochondrial layer well formed, and the break comes across this layer.

9. Unusual breaking into two types in the same batch of eggs: (1) small white and large red halves at left and center; (2) large white and small red half at right. Nucleus shows well in some of these eggs.

10. Lower magnification of white halves of two distinct sizes, no intermediates, from such a batch as shown in Photograph 9. The larger ones appear darker because of the mass of granules; these obscure the oil cap, which is plainly visible in the smaller type.

42. Plutei and blastulæ from whole and white half eggs, fertilized, 2 days old. Several white halves have developed into plutei half the normal size. Other white halves are still spherical blastulæ. (Low magnification.)

43. Parthenogenetic merogone from yolk quarter, 4 weeks old. Detailed drawing of same in Fig. 20, Plate II. Highly magnified.

44 and 45. Fertilized merogones. 44. Fertilized yolk quarter with 2 daughter nuclei. Cell division only indicated by notch at left. Three hours after fertilization. 45. Fertilized red half with about 12 nuclei and no cell boundaries. Five hours after fertilization.

fuging and using more of the sucrose solution, the red halves are often broken into quarters and there are five distinct layers in the tubes: (1) white halves, (2) elongate whole eggs, (3) yolk quarters, (4) red halves, (5) pigment quarters at the bottom (Photograph 6, left). One can decant off from the tubes the two upper layers of cells, which contain nuclei, and obtain a pure culture of non-nucleate fractions. Thus is obtained the material for the study of parthenogenetic merogony, and it can be obtained in great abundance. The study has been made almost exclusively on living eggs and all photographs and drawings are from living material. I have not yet worked with the granular quarters of the white halves; these are more difficult to obtain as it requires prolonged centrifuging. The yolk quarters have proved to be excellent material for the observation of cytological details in the living eggs, owing to their lack of pigment. Even the pigment quarters, though only about one-fourteenth the volume of the whole egg, are activated and cleave.

Parthenogenetic Agents

For parthenogenetic agents, I have used, for the most part, hypertonic solutions: sea water concentrated by boiling to half its volume, or sea water brought to a similar hypertonicity by the addition of NaCl in the proportion of 30 grams of NaCl to a liter of sea water. The addition of KCl to sea water (39 grams to a liter) is efficacious but renders the surface of the egg sticky. Also leaving the eggs in the sucrose solution for an hour or keeping them for 24 hours at 8° C. will initiate development. The eggs are left in the hypertonic solution for twenty minutes, and then returned to sea water. This procedure causes development in both nucleate and non-nucleate fractions, but the different fractions, and also the whole eggs, from the same batch react quite differently. The same solution that activates the red halves may fail to activate the white halves or the whole eggs, and usually when the white halves develop the red halves do not. The batch, however, runs quite uniformly; if some of the red halves are activated, the majority of them are; it is not sporadic. Stretched eggs are activated more readily than unstretched, probably because the membrane is thinner and materials can pass through more easily. The stretched whole egg is activated more readily than the normal uncentrifuged egg, and elongate fractions are activated more readily than the same fractions which have become spherical on standing. The eggs are treated, therefore, immediately after removal from the centrifuge. It may be that the sucrose solution in which the eggs are centrifuged helps the activation.

Development of Non-nucleate Fractions (Parthenogenetic Merogones)

The first sign of activation of any of the centrifuged fractions or whole eggs takes place while in the hypertonic solution; it is a "setting" of the egg. The egg retains its elongate shape and the granules do not redistribute. This is characteristic also of centrifuged whole eggs *fertilized* immediately after centrifuging; they retain their dumb-bell shape and the fertilization membrane follows the irregular contour of the egg (E. B. Harvey, 1932). If, however, they are not fertilized immediately after removal from the centrifuge, but are left unfertilized in sea water, they become spherical within an hour and the granules redistribute. Centrifuged whole eggs and nucleate fractions when activated artificially are also "set."

The non-nucleate fractions are likewise "set" (Photograph 11). In the best batches nearly 100 per cent are "set." Then within ten or fifteen minutes, they usually throw off a fertilization membrane and the ectoplasmic layer forms. In some batches, the fertilization membrane does not form, but development proceeds nevertheless. If kept in the hypertonic solution, the eggs remain in the same condition for some hours with no further development.

After treatment with hypertonic sea water for 20 minutes, the non-nucleate eggs are returned to sea water. Frequently the change from the hypertonic solution to the sea water causes a rupture of the fertiliza-

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Explanation of Plate II

Detailed camera lucida drawings of non-nucleate egg fractions (parthenogenetic merogones) from living material. The coarse stippling denotes yolk granules, the large solid dots denote pigment. (The times given are times after activation and return to sea water.)

Fig. 10. Formation of clear area (astrosphere) in non-nucleate yolk quarter. One hour after return to sea water.

Fig. 11. Formation of monaster in non-nucleate yolk quarter. Two hours after return to sea water.

Fig. 12. Formation of amphiaster.

Figs. 13, 14. First cleavage.

Fig. 15. Non-nucleate red half with many asters and no cell boundaries. "Crater blastula." Still within fertilization membrane.

Fig. 16. Cell boundaries have come in around the asters.

Fig. 17. Embryo emerges from the fertilization membrane as individual cells which later disintegrate.

Fig. 18. Three-day non-nucleate blastula from yolk quarter; large cells inside, small cells outside.

Fig. 19. Eight-day non-nucleate embryo from red half, vacuolated. Not divided into cells.

Fig. 20. *A*. Four-week non-nucleate embryo from yolk quarter, not divided into cells, but with vacuoles and large spherical inclusions. *B*. More detailed drawing of the surface, showing possible cilia. Photograph 43 is of this same embryo, still living.

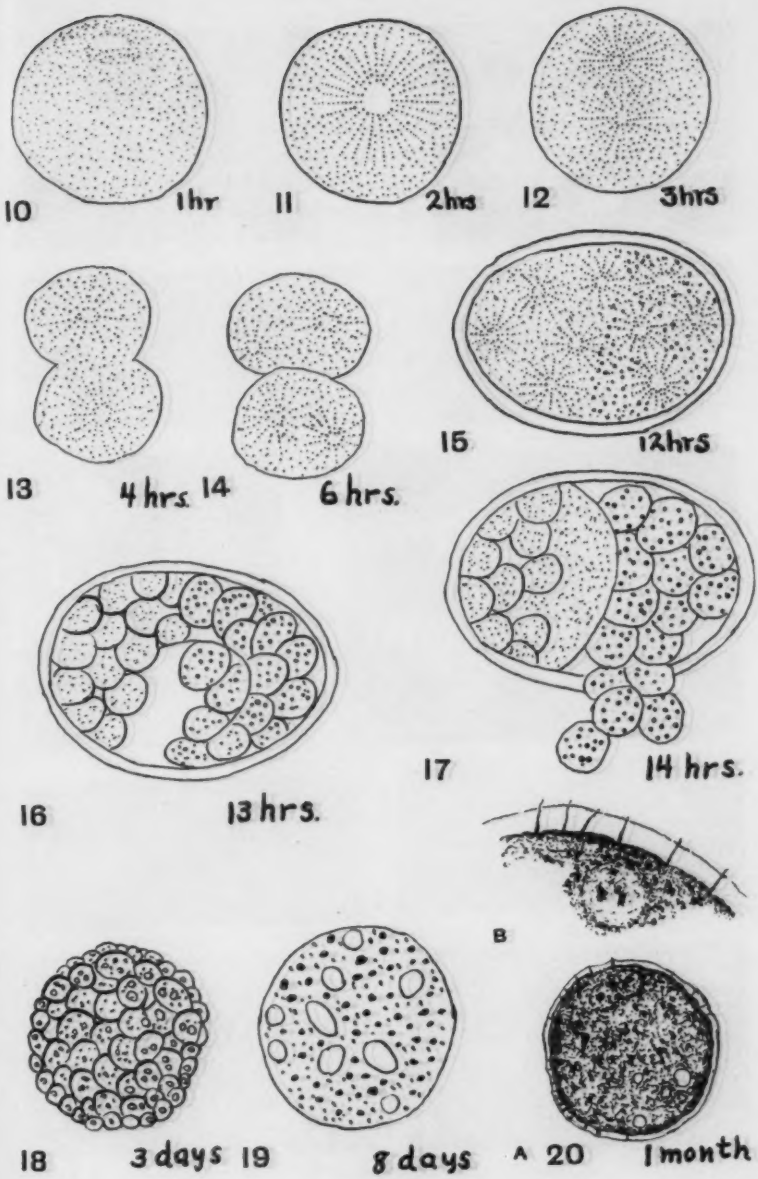
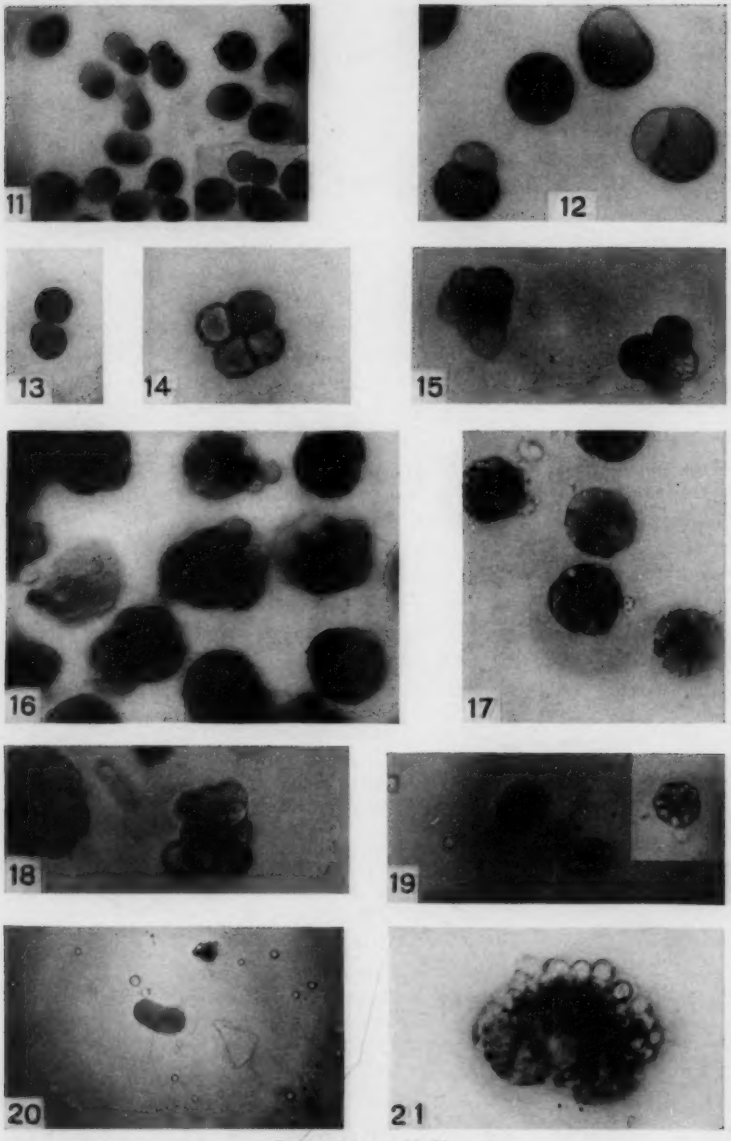

10
1hr
11
2hrs
12
3hrs
13
4 hrs.
14
6 hrs.
15
12hrs
16
13 hrs.
17
14 hrs.
B
18
3 days
19
8 days
A 20
1 month

PLATE II



PHOTOGRAPHS 11–21

All are of living non-nucleate fractions (parthenogenetic merogones)

11. One- and 2-cell stage of red halves; two cells nearly equal in size. Five hours after activation and return to sea water.

tion membrane, owing to the swelling of the egg. After about an hour, a clear area may be observed in the living eggs, especially in the yolk quarters and in the yolk section of the red halves; this is an astrosphere, the beginning of a cytaster (Plate II, Fig. 10). After another hour an aster is formed around this, forming a large and quite typical monaster (Fig. 11). After another hour one often observes two cytasters (Fig. 12), and this may be followed by a cleavage plane between the two asters in quite typical fashion (Figs. 13, 14). A spindle is not visible in the *living* material in these non-nucleate eggs nor indeed in any mitotic division of fertilized eggs. A thorough study of prepared and sectioned material has not yet been made, but a survey of a few slides has failed to reveal anything but asters in the parthenogenetic merogones; these are very granular along the rays.

The activated non-nucleate fractions may divide into two equal or unequal cells (Photographs 11, 12, 13). In the best batches, about eighty per cent cleave. The cleavage plane in the red halves is not constant in position; it often comes in along the boundary between yolk and pigment, but this is not always so. It usually comes in across the narrow axis of an elongate egg, but often not the shortest axis. Very frequently, the egg is pinched in by the free edge of the now hardened fertilization membrane which had broken (usually at the centripetal pole) when the egg was taken from the hypertonic solution to sea water; the cleavage plane usually comes in above this constriction (Photographs 26, 36). This gives a very striking and characteristic 2-cell stage. Such a 2-cell stage is found also in elongate whole eggs treated parthenogenetically (Photograph 31), and also in elongate whole eggs fertilized immediately after centrifuging (Photograph 30). In the latter case, however, the constriction is the original narrowed portion

12. One- and 2-cell stage of red halves; two cells unequal; fertilization membrane present. Five hours after activation.

13. Two-cell stage of yolk quarter. Six hours after activation.

14. Four-cell stage of yolk quarter. Asters faintly visible, one in each cell. Five hours.

15. Six- and 8-cell red half. Four hours.

16. Eight- to 16-cell red halves. Five hours.

17. Eight- to 16-cell red halves. Cells still confined within fertilization membrane. Eight hours.

18. About 24-cell red half. Sixteen hours.

19. About 64-cell red half. Blastula in center breaking through the fertilization membrane, others still enclosed. Twelve hours.

20. Yolk quarter (not divided into cells), emerging from the fertilization membrane in amœboid fashion. Empty fertilization membrane at right. One and one-third days.

21. Yolk quarter, divided into many cells; about 500 in number. Two days.

of the egg preceding the break into two spheres which would have occurred with further centrifuging; it is not caused by a broken fertilization membrane as in the parthenogenetic eggs. In any of these cases, however, it is more usual for the cleavage plane to come in above (centripetally to) the constriction and not through it, determined in all probability by the stratification. The separation between the two non-nucleate cells is a bona-fide cleavage plane and not merely a pinching in of the surface, as is evident from Photographs 26 and 27—the latter taken two minutes after the former.

A second cleavage plane may divide the two non-nucleate cells into four, again between two asters (Photograph 14; asters are visible in Photographs 36 and 14), or it may divide only one of the first two blastomeres, giving a 3-cell stage. Or the non-nucleate egg may occasionally divide at once into 3 or 4 cells. By subsequent cleavages more cells are formed, and there occur fairly regular 8-, 12- and 16-cell stages, and intermediate numbers. Sometimes these are still enclosed in the fertilization membrane (Photograph 17); sometimes they form a loose cluster of cells not confined within a membrane (Photographs 15, 16, 18, 24, 25, 28, 29, 37, 38). Cell boundaries sometimes disappear and new cell boundaries are formed. I have not found any indication of micromeres at the 16-cell stage of the non-nucleate egg, but these apparently do not form even in the whole eggs fertilized immediately after centrifuging, where the cleavage is quite irregular owing to the shape of the egg. The cleavages of the yolk quarters are more regular than those of the red halves, probably because they lack the heavy red pigment and are more homogeneous and more nearly spherical. The pigment cells of the red halves are usually slower to divide than the yolk cells and are therefore, in general, larger. These same facts are true also of similar fertilized fractions.

Even the pigment quarters, though only about one-fourteenth the volume of the whole egg, will cleave when activated. They often divide

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EXPLANATION OF PHOTOGRAPHS 22–29

All are of living non-nucleate fractions (parthenogenetic merogones)

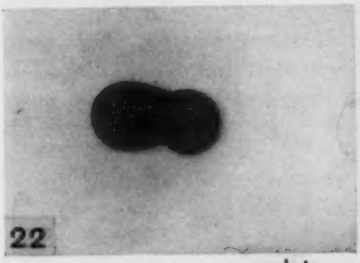
22–25. Series from one and the same red half egg. Times denote hours after activation and return to sea water.

26. Red half 1½ hours after activation. First cleavage plane (at left) beginning through shorter axis. The constriction at right is caused by the pinching in of the broken and now hardened fertilization membrane, and is not a cleavage plane.

27. Same egg 2 minutes later, showing further progress of cleavage plane.

28. At right, 3-cell stage of pigment quarter. At left, about 8-cell stage of red half. Six hours after activation.

29. At right, below, 4-cell stage of pigment quarter. Above, about 6-cell stage of red half. Six hours after activation.

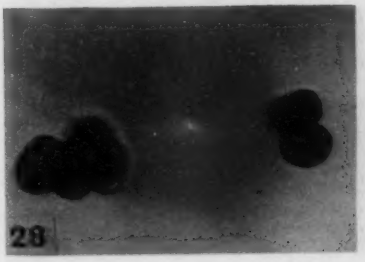


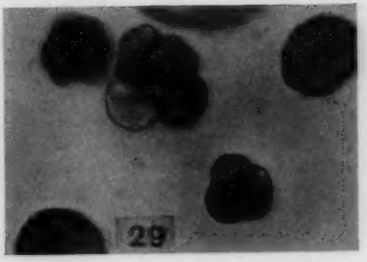
$1\frac{1}{2}$ hrs.

4 hrs.

5 hrs.

$5\frac{1}{4}$ hrs.





PHOTOGRAPHS 22–29

into two equal cells, and by subsequent cleavages into as many as 16 cells (Photographs 28, 29, 37). These unfertilized activated quarters have in my experiments cleaved better than the fertilized pigment quarters; I have obtained the latter with 16 nuclei but without cell boundaries.

It should be especially noted that cleavage follows upon cleavage in the non-nucleate egg in a fairly regular sequence. Photographs 22–25 are a series from the same egg at various intervals. The cleavages are not so regular as in normal uncentrifuged fertilized eggs, where they occur with clock-like regularity, but they are almost as regular in occurrence as in fertilized or parthenogenetic centrifuged eggs and in fertilized fractions. Indeed the similarity of the cleavage figures in the non-nucleate egg fractions to those in nucleate ones or in nucleate whole eggs is very striking. The cleavage of a centrifuged egg fertilized immediately after centrifuging is different from that of a normal fertilized egg owing to its elongate shape and to the uneven distribution of granules. The cleavage of an elongate stratified non-nucleate egg fraction is precisely like that of a similar nucleate fraction. Whether

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EXPLANATION OF PHOTOGRAPHS 30–41

Two vertical columns arranged for comparison of nucleate (30–35) with non-nucleate (36–40) eggs at similar stages of development.

30. Two-cell stage of whole egg fertilized immediately after centrifuging. One hour after fertilization.

31. Two-cell stage of whole egg, unfertilized but artificially activated immediately after centrifuging. Four hours after activation.

32. Below, 2-cell stage of pigment quarter. Above, 8-cell stage of yolk quarter. Three hours after fertilization.

33. Sixteen- to 32-cell stage of whole eggs 3 hours after fertilization. At right, the two components from the first two cells have developed separately without fertilization membrane; with further development these would produce twins.

34. Early blastulæ 4 hours after fertilization. At left, whole egg. At right, white half, unusually large. Still within fertilization membranes.

35. Late blastula from yolk quarter, 1 day after fertilization.

36. Two-cell stage of non-nucleate red half. Note two asters in upper cell. Six hours after activation.

37. Above, at left, 8-cell stage of non-nucleate yolk quarter. Above, center, 2-cell stage of pigment quarter. Below, right, 8-cell stage of red half. All non-nucleate. Five hours after activation.

38. Sixteen- to 32-cell red halves, non-nucleate. Nine hours after activation. At right, the two components from the first two cells have developed separately; with further development these would produce twin blastulæ.

39. Early non-nucleate blastulæ, 12 hours after activation. At left, red half. At right, yolk quarter. Still within fertilization membranes.

40. Late non-nucleate blastula from yolk quarter, 2 days after activation.

41. Left, pluteus from whole egg, 3 days after fertilization. Right, above, small blastula from non-nucleate yolk quarter, 3 days after activation. To show comparative size and differentiation after 3 days.

42–45 follow Photograph 10.

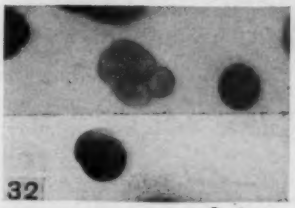
NUCLEATE

NON-NUCLEATE

Fertilized
1 hr

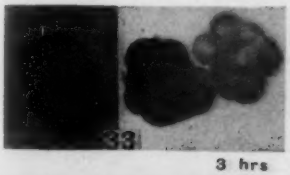
Parthenogenetic
4 hrs

Parthenogenetic Merogone
6 hrs



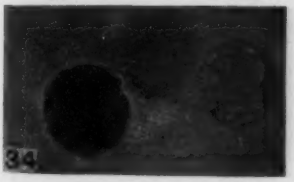
3 hrs

5 hrs

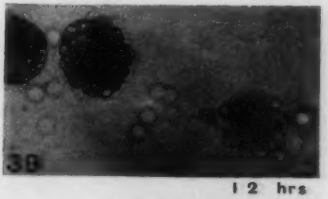


3 hrs

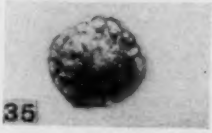
9 hrs



4hrs

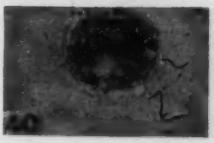


12 hrs



1 day

3 days



2 days

PHOTOGRAPHS 30–41

the nucleate egg or fraction has one or two nuclei, that is, whether it is fertilized, or parthenogenetic, or merogonic, makes little difference in the type of cleavage. Compare, for instance, the 2-cell stage of a fertilized whole egg (Photograph 30), a parthenogenetic whole egg (Photograph 31), and a non-nucleate red half (Photograph 36). The first cleavage, as mentioned previously, in an elongate egg usually comes in across the shorter axis in the less dense area. Later cleavages of nucleate and non-nucleate eggs are closely similar, as a comparison of Photographs 32–35 with those of Photographs 37–40 will show. Cleavage stages of nucleate eggs are at the left and the corresponding stages of non-nucleate ones at the right. Even the frequent independent development of the first two blastomeres is similar (Photographs 33 and 38). The main difference is in the rate of cleavage. The non-nucleate eggs cleave much more slowly. Whereas it takes about fifty minutes (at 23° C.) for the first cleavage of a fertilized whole egg, whether normal or centrifuged, and the same time for the fertilized white half, it takes about six hours after activation for the first cleavage of a non-nucleate egg. The times for first cleavage as well as later ones are extremely variable in different sets of the non-nucleate eggs. A nucleate egg, treated parthenogenetically, is intermediate in rate; it takes about four hours after activation for the first cleavage. The merogonic egg fractions, i.e., fertilized enucleate parts of eggs, are also slow to cleave (Photograph 32; see also E. B. Harvey, 1932). In general, eggs with 2 nuclei (♂ and ♀) develop most rapidly, those with one (♂ or ♀) less so and those without any nucleus most slowly; the very slow development of the fertilized clear quarter is an exception (1932). It may be that the nucleus contains an enzyme which accelerates cleavage, but apparently certain granules must be present.

Later cleavages of non-nucleate fractions (parthenogenetic merogones) result in the formation of a blastula. The blastula, if still confined within the fertilization membrane, may be observed breaking through the membrane after about twelve hours (Photographs 19, 39). It frequently happens that cleavage planes do not come in, especially in the dense red halves, and more and more cytasters appear as time goes on, giving the surface somewhat the appearance of the surface of the moon, pitted by many craters (Fig. 15). Exactly the same phenomenon was noted in the development of the fertilized red half-eggs (Fig. 20, 1932), only here nuclear membranes are present. Photographs 44 and 45 are of fertilized enucleate fractions showing a multinucleate condition without cell boundaries. Cleavage planes frequently come in, in these red halves both fertilized and non-nucleate, just before the breaking of the fertilization membrane, thus dividing the egg into many cells

(Fig. 16). The non-nucleate blastula sometimes emerges as isolated cells instead of as a complete organism, and then goes to pieces (Fig. 17). Sometimes the cleavage planes do not come in even at this late stage, and the embryo emerges from the fertilization membrane as a compact mass of protoplasm, somewhat amoeboid in appearance, but perfectly viable (Photograph 20). The oldest cellular non-nucleate blastulæ obtained were from yolk quarters two and three days old (Fig. 18, Photographs 21, 40). These consisted of somewhat differentiated cells, the inside cells larger than the peripheral; the individual cells were filled with granules and small vacuoles or fat droplets. As many as 500 cells have been counted in these blastulæ. Staining *in toto* with aceto-carmine failed to reveal nuclei or chromosome plates which are well brought out in fertilized yolk quarters similarly treated. The embryos usually do not survive more than three or four days owing both to their low vitality and to the accumulation of decomposing eggs in the culture with resultant growth of bacteria and protozoa. Some of the non-cellular embryos which were isolated have survived longer; an eight-day embryo from a red half is shown in Fig. 19, consisting of granular protoplasm filled with large vacuoles and some pigment. The oldest embryo was still viable after a month (Photograph 43, following Photograph 10; and Fig. 20). This, from a yolk quarter, was a mass of protoplasm filled with yellowish granules and small vacuoles and larger spherical inclusions, possibly individual cells. There were fine strands radiating from the protoplasm to and possibly through the thin enveloping membrane characteristic also of normal free-swimming blastulæ; these are possibly short cilia, and the organism may have been slightly motile. Whether a non-nucleate egg can give rise to a true free-swimming ciliated blastula is still uncertain. It can, however, develop into a many-celled embryo having a certain amount of differentiation in that the cells are of different sizes; or into an embryo without cell boundaries but with differentiated protoplasm, perfectly viable a month after activation with parthenogenetic agents. The unfertilized and untreated sea-urchin eggs live only two or three days at room temperature, according to Loeb and Lewis (1902); they certainly do not survive longer than this in mass cultures without special care.

The striking similarity of the nucleate and non-nucleate eggs stops with the late blastula of one or two days (*Cf.* Photographs 35 and 40). I have seen no indication in the non-nucleate embryo of the formation of a gut or of the arms and skeleton characteristic of a pluteus. The normal fertilized egg has developed into a well-formed pluteus in three days, whereas the parthenogenetic merogone is still a spherical blastula. A photograph (41) of one of the 3-day parthenogenetic non-nucleate

yolk quarters together with a pluteus of the same age from a normal fertilized egg shows the disparity in size and differentiation. The parthenogenetic yolk quarter of a month remained the same size with little change, whereas the normal pluteus in that time changes greatly in size and shape. Since the non-nucleate eggs are slower to develop in all stages, it is just possible that with longer time they might develop further than the blastula. Such a possibility is suggested by a comparison of larvæ from fertilized half and whole eggs 2 days old. In Photograph 42 (following Photograph 10) are shown normal plutei together with half-size plutei from fertilized white half-eggs. There are also present many spherical blastulæ of the half-eggs (which are slower to differentiate) not yet developed into plutei. As observation showed, many of these did develop into plutei later on. A comparison of Photographs 41 and 42 would at least suggest that the small blastula of the parthenogenetic merogone might also develop into a pluteus.

Discussion

There has been considerable discussion as to whether cells without nuclei can divide, or whether asters can divide in the absence of chromatin. Boveri (1918) threw doubt himself on his earlier conclusions that asters (but not cells) could divide without chromatin. Dalcq (1931) has reviewed all the pertinent cases and has come to the conclusion that they are all subject to criticism, and that cell division does not take place without chromatin, and this seems to be the case with his own experiments. Fankhauser (1929, 1934) believes, however, that the large number of non-nucleate cells, some with amphiasters and even spindles, in his merogonic *Triton* eggs, indicates their origin from other non-nucleate cells, though he believes that nucleate cells must be also present to influence division. The presence of accessory sperm nuclei in the developing amphibian egg prevents these results from being absolutely clear-cut.

Wilson (1901) was the first to describe multiplication of asters in the entire absence of chromatin in the non-nucleate fragments (obtained by shaking) of *Toxopneustes* treated parthenogenetically, but he found no cell division. His results were questioned by Fry (1925), who maintained that in enucleate fragments accurately cut by hand and treated with parthenogenetic agents, asters might arise and irregular cleavage come in but the asters did not multiply. McClendon (1908), with a primitive micro-manipulator, very ingeniously removed the nucleus from the starfish egg and treated the egg with a parthenogenetic agent. He concluded that cells could divide without chromatin though

the segmentation was quite irregular. These results were not unequivocal since many of the operated eggs contained chromatin.

There seems no doubt from the present observations that cell division can take place without nuclei. I have previously (1935) shown that the nucleus may be moved by centrifugal force from its normal position and cell division come in with no relation to its final position. Cleavage may also come in while the nucleus is still intact, as I noted previously in *Parechinus* (1935, Fig. 11), and as I have recently observed under certain conditions in *Arbacia*. The present work shows that the nucleus does not have to be present at all in cleavage. Asters arise *de novo* in non-nucleate eggs and become more numerous with time. Normal cleavages come in exactly as they do in nucleate eggs of the same sort and shape, and cleavage follows upon cleavage in a fairly orderly fashion.

An egg fragment lacking both maternal and paternal chromosomes has given rise by repeated cleavages to an embryo containing about 500 cells with a certain amount of differentiation. An embryo arising from a non-nucleate egg has lived a month; it is probable but not certain that these have cilia and are free-swimming. The early stages of development can, therefore, take place without chromosomes. This means that the maternal cytoplasm is of great importance and has within itself the potentialities of determining at least the early stages of development. These potentialities must either (1) be given to the cytoplasm by the nucleus previously, or (2) be innate in the maternal cytoplasm and entirely non-nuclear. They might be given to the cytoplasm either at the time of the breakdown of the germinal vesicle, or else earlier by the chromosomes of a preceding generation. The former alternative cannot be tested experimentally since the egg and egg fragments cannot be artificially activated, nor are they fertilizable, before rupture of the germinal vesicle. An influence on egg cytoplasm caused by the chromosomes of a preceding generation has been postulated for spiral twisting in snails and for certain characters in the silkworm and in corn. It would certainly be difficult to prove experimentally in the case of the sea-urchin egg. The second supposition, i.e., that the potentialities of early development are innate in the cytoplasm, restricts inheritance by genes to later developmental stages, and it may very well be that only the more specific and differential characters are controlled by the genes, whereas the general and fundamental characteristics of living matter are cytoplasmic. If further work shows, however, that the development of the parthenogenetic merogones does not stop with the blastula, but that they will develop into plutei, the results will be difficult to harmonize with the accepted ideas of the mechanism of genetical inheritance.

I have shown by previous studies (1932, 1933) that the visible gran-

ules in the eggs of several species of sea urchins are not of great importance in development. Any part of the egg seems capable of development, and many parts have, when fertilized, given rise to quite normal plutei with skeletons. Any difficulty in development seems to be mechanical rather than structural, such as the breaking apart of the blastomeres in the clear quarters of the *Arbacia* egg, owing to the thinness of the membranes; or the absence of cleavage planes in many of the red halves, owing to the very dense granular cytoplasm. No particular type of visible and moveable granules seems essential to development; these must be concerned with metabolism and respiration. It must therefore be the "ground substance" which is the material fundamental for development—the matrix, which is not moved by centrifugal force and which, in the living egg, is optically empty.

Summary

1. Non-nucleate fractions of *Arbacia* eggs obtained by centrifuging can be activated by parthenogenetic agents, and develop; these may be termed "parthenogenetic merogones."

2. Asters are formed and cell division takes place without nuclei.

3. Cleavages of non-nucleate fractions are strikingly similar to those of nucleate fractions (or whole eggs) of the same stage of development.

4. Many cleavages, in sequence, result in the formation of a blastula of as many as 500 cells.

5. Blastulæ emerge from the fertilization membranes; one embryo was still viable after a month.

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SEQUENCE OF FUNCTIONAL SEXUAL PHASES IN TEREDO

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In a recent number of this journal certain questions have been raised by Grave and Smith (1936) as to the sequence of the functional sexual phases and the proportions of individuals in each phase in the successive age groups of the variety of *Teredo navalis* found so abundantly at Woods Hole and other localities on the coast of southern New England. Evidence is now available which will answer these questions with some degree of positiveness.

In his earlier studies on the biology of this species at Woods Hole the senior author (Grave, 1928) stated that the females outnumber the males but he made no mention of the possibility of a change of sex. The recent investigations by Grave and Smith, however, indicate a sequence of alternating phases of functional sexuality comparable with that of certain species of oysters. They thereby confirm in most respects the conclusions reported during the past few years on the sexual rhythm of this species (Coe, 1933*b*, 1934*a*, 1935). Yet in regard to the proportions of individuals in each of the successive phases there is some divergence of opinion and it will be shown on the following pages that the reason for this discrepancy may be partly due to the failure of Grave and Smith to follow the life cycle through the entire sequence of sexual phases. Their published investigations cover only the two months from June 28 to August 27, 1935.

It is quite illogical to treat the sperm-producing and egg-producing phases of the teredo as distinct categories of individuals, since they represent merely successive stages in the life of a single individual. If the number of older animals in the functional female phase is found to be twice as great as in the alternate phase of sexuality, the explanation may be simply that the duration of one sexual phase averages twice as long as the other, always assuming that no selection, intentional or otherwise, as to ages is involved. Perhaps an exception should be made in regard to the relatively few individuals known as true males, in some of which the initial sexual phase may be retained for an indeterminate period. In still fewer cases the individual appears to be proterogynous.

Protandry in *Teredo* has been indicated, although not recognized as

such, since the work of Quatrefages nearly a century ago, for he estimated that there were about twelve times as many females as males in the adult population. Nelson later suggested an even greater disparity of the sexes, with as many as five hundred females to one male among large individuals. Yonge (1926), on the other hand, found definite evidence of protandry in two specimens of *T. norvegica,* while Sigerfoos (1908) had previously suggested protandry in *Bankia gouldi.*

Substantial proof of a sequence of sperm-producing and egg-producing phases during the lifetime of the same individual has been published in the series of recent articles to which reference has been made (Coe, 1933*a*, 1933*b*, 1934*a*, 1935). In the first of these papers it was reported that an actual transition from the functional male phase to the functional female had been observed in about half of the small individuals infesting ropes in New Haven Harbor. The second paper (Coe, 1933*b*) gave detailed descriptions of the transformations of the primitive bisexual gonad of the very young individual, through the initial male phase and thence to the female phase toward the end of the breeding season. No evidence was available at that time that the female phase might again revert to the male condition but this transformation of sex was reported, with details of the cellular changes involved, in the third paper of the series (Coe, 1934*a*).

In a more recent brief summary of the sexual changes which the several types of individuals are believed to undergo the successive phases were schematized (Coe, 1935) as follows:

1. Male . . . female . . . recuperation . . . female.
2. Male . . . female . . . recuperation . . . male . . . female.
3. Male . . . recuperation . . . female.
4. Male ('true male') . . . recuperation . . . male . . . (female?).
5. (Exceptional.) Female . . . recuperation . . . (male?).

To this series the paper by Grave and Smith (1936) adds one additional aspect, namely, that the transformation from female to male phase may occur abruptly during the activities of the breeding season as well as in the recuperation period.

Proportion of Individuals in Each Functional Sexual Phase at Successive Ages and at Different Seasons

The accompanying Tables I and II indicate the sexual condition of 2,599 individuals grouped according to their sizes and approximate or maximum ages at each season of the year. The gonads of about three hundred of these were cut and mounted as serial sections; for each of the others the gonad was teased and examined microscopically. Most

of the collections were made at Woods Hole, Massachusetts; others were obtained from Milford, Connecticut, through the courtesy of Dr. Victor Loosanoff, and a few from Barnegat Bay, New Jersey, through the kindness of Dr. T. C. Nelson.

Examination of 388 additional large individuals during the third week in June, 1936, showed 29 true males, 81 bisexual males, 17 hermaphrodites, 95 females with nearly ripe ova, and 166 females carrying embryos. Omitting the hermaphrodites, these numbers would represent a population having 29.65 per cent of the adults in the functional male phase, with 70.35 per cent functioning as females at the time of the examination. These data were obtained too late to be included either in the graph or in Table II, but they would not have changed greatly the percentages there indicated except for the larger proportion of females with embryos.

Since the sexual phases of most individuals change progressively with their advance in age, it is obviously impossible to classify them with great accuracy. Furthermore, there are so many individual differences in the expression of the male and female characteristics that the difficulty is greatly increased. Even if all individuals with ripe spermatozoa are classed as functional males, there are always found in a large collection some with a few sperm associated with many ova. Still more difficult and uncertain is the separation of functional males into the two groups of true males and bisexual (hermaphroditic) males, since all grades occur in the relative size and abundance of the ovocytes which even in many of the true males can be detected along the walls of some of the follicles.

Nor can the precise age of any individual be determined with accuracy because the teredo will not live and grow normally outside the wood in which it feeds. The maximum age is determined by reckoning from the time when the wooden block is placed in the water during the breeding season. The size is not strictly a reliable criterion of age, since rapidity of growth depends so largely on environmental conditions. The characteristics of the shell and of the pallets are sometimes helpful.

With these limitations in mind the data in Tables I and II will show the seasonal trend of the successive broods and the sequence of sexual changes which they undergo from immaturity to advanced age.

Attention may first be directed to the group of 43 individuals shown in Table I to have been collected on July 19 from a block which had been in the water for only 20 days. In this brief period 24, or more than half the total number, had already reached the functional male phase, while one only was producing ovocytes exclusively. The 18 individuals which were sexually immature were evidently still younger and later arrivals.

It thus appears that there were in this lot 24 protandric individuals as compared with one which was proterogynous. The evidence from the entire series of 911 small teredos listed in Table I is consistent with the conclusion that the first sexual phase is, with few exceptions, masculine.

The gonads of these young individuals, however, form an intergrading series, from those which have only a few small ovogonia or ovocytes on the walls of the follicles (true males) to those which are so strongly feminine that they consist mainly of large ovocytes, with relatively few spermatogenic cells in the lumens. All except the true males are grouped in Tables I and II as bisexual males.

It will be noted that more than 10 per cent of the small teredos listed were in the female phase. Presumably most of these were dwarfed animals that had already completed a brief initial male phase rather than strictly proterogynous individuals.

The second sexual phase, which immediately follows the initial male phase except in the autumn, is usually feminine. True males, however, may resume and continue spermatogenesis for an indeterminate period.

No proof is at present available that a third sexual phase occurs in these localities during the same year. More frequently a long recuperation period follows the completion of the female phase in late summer and autumn. During the winter the gonad of such individuals is reorganized and the third sexual phase, which may be of either type of sexuality, develops during the late winter and early spring. The breeding period lasts from about the middle of May to the middle of October.

The over-wintering teredos will thus include individuals of all ages, from those young ones which settled on the blocks in the autumn and are still sexually immature to those which completed at least two sexual phases the preceding breeding season. The most numerous group, however, will be of the younger ages.

In the pre-spawning season (Table II) the two alternate sexual phases are found in about equal numbers, or with a small majority in the female phase. Some of the individuals classed as being in the bisexual male phase evidently have but a brief period of sperm production at the beginning of the second spawning season, for the ratio of animals in the female phase appears to increase with advancing age (Table II). The percentages in each sexual phase are shown graphically in Fig. 1. It is evident either that the number of animals changing from male to female phase is greater than the number going in the opposite direction or that the female phase is of longer duration than the alternative aspect of sexuality.

The data compiled by Grave and Smith (1936), on the other hand,

would indicate that the ratio of animals in the female phase decreases during the summer from approximately 3 to 1 (150–58) in June to 46–55 (65?) in August, or about 1 to 1 (58–55) if the hermaphrodites are included among the females. The differences between these data and those here recorded may be due in part to chance selection and in part to differences in classification. In many cases the bisexual gonad shows such an intermediate condition between male and female phases as to make any distinction more or less arbitrary. Moreover, shortly after the middle of August the first young of the year may attain a length of 100 mm. or more—a size sufficient to be classed as "large specimens." If some of these were to be included with the older individuals there would appear to be a rapid increase in the male-phase ratio.

TABLE I

Sexual phases of small individuals (20–60 mm. in length) of approximately known ages

Date	Maximum age	Sexually immature	True male phase	Bisexual male phase	Young female phase	Mature female phase	Females with embryos	Total
	days							
Mar. 29	—	0	4	18	0	0	0	22
May 19	—	0	5	21	20	0	0	46
June 25	—	0	5	29	13	7	0	54
July 19	20*	18	6	18	1	0	0	43
Aug. 18–23	71	8	71	415	6	4	3	507
Sept. 19–24	112	0	5	80	16	12	6	119
Sept. 30	42	41	0	12	1	0	0	54
Nov. 1	73	16	11	32†	5	2	0	66
Total		83	107	625	62	25	9	911
Per cent		9.11	11.75	68.61	6.80	2.74	0.99	

* From Barnegat Bay, N. J. Courtesy Dr. T. C. Nelson.
† Winter condition.

It is, however, in the late spawning season that the greatest preponderance of large individuals in the female phase is found; for instead of the former ratio of about 2 to 1, a collection of 164 large individuals in September gave a female ratio of approximately 10 to 1 (not counting 7 hermaphrodites), as shown in Table II and Fig. 1. These animals, although only 3 to 4 months of age, had already reached a length of more than 100 mm. and had, with relatively few exceptions, already completed the initial male phase. By selecting a group measuring 70–95 mm. in length, some of the younger, male phases were included and the ratio approximated equality (Fig. 1). Selection of 119 still smaller and younger animals on the same dates showed more

TABLE II

Sexual phases of large individuals (90–275 mm. in length)

Date	Maximum age, months	True male phase	Bisexual male phase	Hermaphrodites	Unripe female phase	Mature female phase	Females with embryos	Spawned out	Total
	Pre-spawning Season								
Apr. 20–25	7–10	11	66	0	48	81	0	0	206
29	7–10	0	33	0	16	0	0	0	49
30	7–10	3	21	0	30	0	0	0	54
May 10	8–11	8	32	0	5	9	1	0	55
Total		22	152	0	99	90	1	0	364
Per cent		6.05	41.76	0	27.16	24.73	0.3	0	
	Early Spawning Season								
May 19	8–11	7	35	0	10	11	11	0	74
25	8–11	4	35	0	11	14	12	0	76
June 7–13	9–12	11	12	0	32	57	17	0	129
16	9–12	3	18	0	7	24	6	0	58
24–26	9–12	7	39	0	18	51	11	0	126
Total		32	139	0	78	157	57	0	463
Per cent		6.91	30.03	0	16.85	33.90	12.31	0	
	Middle Spawning Season								
July 23	12–14	5	1	0	0	5	22	0	33
Aug. 18–21	2–3	18	60	3	0	82	57	0	220
Total		23	61	3	0	87	79	0	253
Per cent		9.09	24.1	1.19	0	34.40	31.20	0	
	Late Spawning Season								
Sept. 19–24	3–4	9	5	7	0	52	84	7	164
Per cent		5.5	3.0	4.3	0	31.7	51.2	4.3	
	End of Spawning Season								
Oct. 3–6	4	2	5	0	4	13	0	94	118
7–8	4	8	11	0	0	23	9	41	92
Nov. 1	2–3	0	0	0	0	6	0	73*	79
13	3	0	0	0	0	0	0	65*	65
15–17	5–6	3	17	0	0	0	0	70*	90
Total		13	33	0	4	42	9	343	444
Per cent		2.92	7.42	0	0.90	9.45	2.03	77.25	
Total all seasons		99	390	10	181	428	230	350	1688
Per cent		5.86	23.10	0.59	10.72	25.35	13.62	20.74	

* Winter condition.

than twice as many functioning as males as were in the alternate phase of sexuality, and the proportion of sperm-bearing individuals in the total population was still greater, since the number of young animals greatly exceeded that of the older individuals in the female phases.

Toward the end of the breeding season, when most of the older individuals have spawned out there is again an approximately equal number of the large animals in each sexual phase (Table II; Fig. 1). Incom-

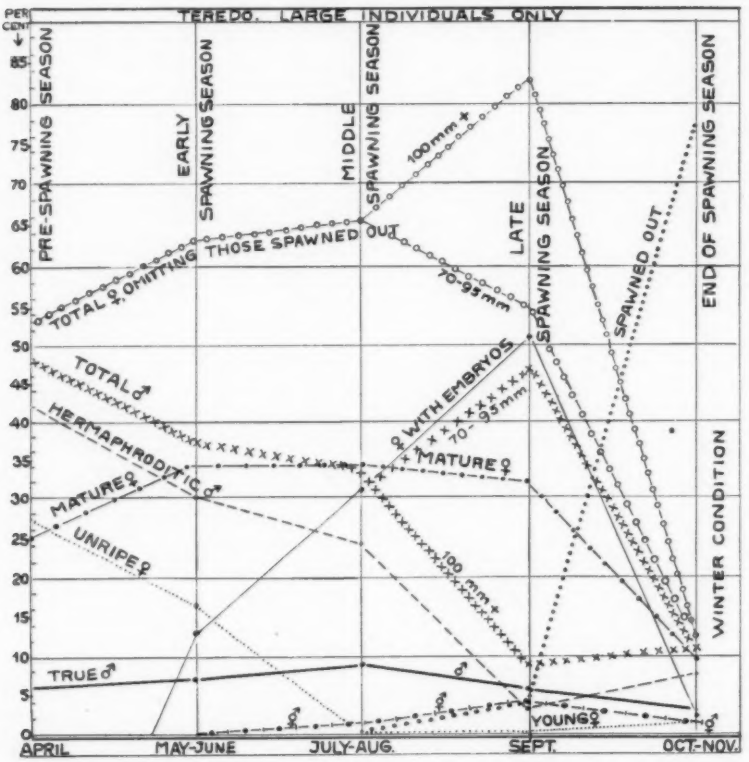

FIG. 1. Graphs showing the proportions of large individuals (90–275 mm. in length) in each phase of sexuality before, during and at the end of the breeding season. Based on a total of 1,688 individuals. It is to be noted that the chart includes two generations of animals, the over-wintering individuals being supplanted by the new broods which attain the "large" size after the middle of the spawning season. The graphs indicating total male and female phases in the late spawning season differentiate two classes of individuals, namely, those 70–95 mm. in length and those of still larger sizes.

pleted sexual phases may be retained during the winter, but most of the partially developed sexual cells will be absorbed by phagocytosis.

The Normal Life Cycle

Turning now from the statistical study of selected groups of animals to a consideration of the sequence of sexual phases realized during the normal lifetime of the single individual, it becomes evident that several different influences are involved. Examination of the gonads of animals of approximately known ages shows clearly the protandric nature of the vast majority of all individuals. Ripe spermatozoa may be produced within three to five weeks after metamorphosis.

The ensuing phase is predominantly female, the percentage of females being influenced by the season when hatched and presumably by hereditary factors as well as by the environment, since all grades of bisexuality appear simultaneously. Eggs may have been liberated and embryos found in the gill chambers within six weeks after metamorphosis. There is no doubt of the strong tendency toward a series of alternating phases, the number of such phases that may be realized by any individual depending largely upon its length of life. At least four alternating phases may occur—two in each of two breeding seasons, but it is not improbable that the number may occasionally exceed four.

The cooler water of early autumn checks the reproductive activity of such individuals as have not already ceased gametogenesis for the season and entered upon the recuperative phase. Most of the oldest individuals are killed by parasitic Protozoa about the middle of the breeding season, as noted by Grave and Smith (1936). The epidemic later affects the earlier broods of the same year, causing the death of many individuals not more than three to five months of age.

The gonads of the survivors, including the spawned-out individuals as well as the others, are reorganized during the winter, in many cases with a change in the sexual phase. Disintegrating residual cells from the previous phase of sexual activity are removed by phagocytosis before the proliferation of the definitive germinal cells.

True Male Phase

This phase represents the extreme aspect of masculinity in that the gonads have only a very few ovogonia or minute ovocytes along the walls of the follicles. Such individuals form but a small proportion of the youngest sexually mature animals (Table I). They occur also as the third sexual phase, following the discharge of the ovocytes of the female phase, but appear to be indistinguishable from those which retain the initial male phase after a long period of growth (Table II).

Individuals of this type were observed by Grave and Smith (1936), who conclude that a "careful study of adult individuals leads us to doubt if there is such a thing as a true male or a true female *Teredo* in the sense that it remains permanently one sex, as postulated by Coe, who agrees that sex inversion in true males may ultimately take place." The two principal parts of this sentence are obviously contradictory, and the word "permanently" has been substituted for "indefinitely" which was used in the papers under discussion. Otherwise there would have been no ground for disagreement, for "true males" are defined in those papers (Coe, 1933*b*) as individuals in which "the preliminary male phase is of longer duration and the crop of spermatozoa is much larger."

The fact that such individuals are found at earliest sexual maturity as well as later is indicative of the operation of genetic factors which differentiate them from others of the same brood. The latter reveal by their gonads a more feminine tendency and are classed as bisexual males or protandric females. This appears to be equally true of other mollusks in which a similar type of sexuality has been reported (Coe, 1934*b*, 1936).

Bisexual Male Phase

The majority of young individuals on reaching sexual maturity are obviously protandric, with gonads showing a highly variable proportion of spermatogenic and ovogenic cells. During the recuperation period there is frequently a return to a similar condition (Tables I and II, Fig. 1).

Functional Bisexuality

Most of the sexually mature teredos examined during the breeding season were actively producing either sperm or ova, but a few individuals occur, as Grave and Smith (1936) have reported, which contain functional gametes of both types. Only 10 of these hermaphrodites were recognized among the 2,599 animals examined (Table II).

In other cases spermatogenesis is well advanced while embryos are present in the gill chambers. Moreover, in an occasional individual some follicles of the gonad may be producing spermatozoa while adjacent follicles contain nearly ripe ova. Such hermaphrodites would not of themselves be indicative of a change of sex in either direction. Of the 618 sexually active individuals reported by Grave and Smith, 27 were classed as hermaphrodites. A collection of 388 adults from the Eel Pond at Woods Hole examined during the third week in June, 1936, contained 17 individuals in this phase of sexuality.

Proterogyny

The finding of an occasional individual in the female phase at earliest sexual maturity indicates that the initial male phase may sometimes be aborted or omitted, as it is known to be in some other species of normally protandric mollusks (Coe, 1936).

Post-spawning Phase

The "spawned-out" condition which may lead again to the bisexual phase or to either of the unisexual phases during the period of recuperation appears to follow the female phase more often than otherwise, although a similar condition results when spermatogenesis ceases and the sperm are discharged without a reciprocal growth of ovocytes. This phase occurs principally toward the end of the breeding season (Table II, Fig. 1), but is also found whenever gametogenesis ceases from any cause.

Summary

Examination of the gonads of 2,987 specimens of *Teredo navalis* of all ages, at all seasons, and during three successive years supplements the conclusions previously published regarding the sequence of sexual phases in this species. The functional phase, if any, in which any given individual is found will, in general, depend upon: *a*, age; *b*, size; *c*, season; *d*, season when hatched; *e*, its particular combination of genetic factors.

Each normal individual shows a tendency toward an alternating series of functional sexual phases. There is good evidence that at least four of these phases may sometimes be completed in a lifetime of less than two years, although the average individual experiences hardly more than one or two.

Among the variable sequences of phases which different individuals may experience the following may be considered typical:

1. Male . . . female.
2. Male . . . female . . . male . . . female.
3. Male . . . female . . . male.
4. Male . . . female . . . female.
5. Male ("true male") . . . male . . . female.
6. Male ("true male") . . . male.
7. Female (exceptional) . . . male . . . female.

Because the population consists of a vastly greater number of individuals of the younger ages than of the older age groups, it is obvious

that death by suffocation, starvation, parasitization or some other cause will usually terminate the individual's existence before the normal cycle is completed.

Protandric bisexuality is generally characteristic of the early sexual phases, with a tendency toward alternating unisexuality in later life. Of the latter, the female phase is usually of longer duration than the alternate male phase.

The proportion of individuals in each sexual phase in the general population or in selected groups will obviously be correlated with a combination of the conditions mentioned above, but limited by associated environmental influences. The total population of teredos living in a particular block of wood may thus consist of upwards of 90 per cent of individuals in either functional sexual phase or of about equal percentages of each, according to the age group or groups represented at the time of the examination.

Attention has been previously (Coe, 1934*a*) called to the similarity of the sexual rhythm in *Teredo* to that of the larviparous oysters, "although the usually shorter life of the former limits the number of the alternating sexual phases." There is some resemblance also to the sexual changes in the oviparous oysters (Coe, 1934*b*) and in the hard-shell clam *Venus* (Loosanoff, 1936), which are predominantly, but not exclusively, protandric, with a later change to the seasonally unisexual condition of the older individuals, in which there is an approximate equality in the sex ratio.

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THE OCCURRENCE AND SIGNIFICANCE OF NITRITE IN THE SEA

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In studying the nitrogen cycle in the sea a consideration of the nitrite concentration in the water is especially useful, because of its intermediate position between ammonia on the one hand and nitrate on the other. Although it is not a stable end-product, its very instability makes it a possible indicator of the state of equilibrium between the oxidation and reduction processes making up the cycle. Its order of magnitude in the water is much smaller than that of nitrate, or even ammonia, but the methods for its analysis are correspondingly more accurate, so that its normal variations may be followed as closely as those of the other nitrogen compounds.

In view of the ease with which nitrite determinations may be made, it is surprising that there are not more data on its occurrence in sea water. Raben (1914, 1918) and Brandt (1927) were among the first to report such figures, but their data were only scattered and fragmentary. Orr (1926) reported some analyses of samples from the vicinity of the Clyde Sea, made observations on diatom cultures, and discussed the factors concerned in the appearance and disappearance of nitrite. Harvey (1926) failed to find significant amounts of nitrite in the English Channel or in deeper waters of the Atlantic further south, but noticed their occasional appearance in stored samples. Atkins (1930), however, was able to make a fairly comprehensive study of the seasonal changes in nitrite in the English Channel, finding high concentrations at certain levels and at certain times. Orton (1924), investigating various influences upon the growth of oysters, found moderately high concentrations in samples from Plymouth Sound, Thames Estuary and Helford River. He also followed nitrite changes in the water of storage tanks.

In a few cases, figures for nitrite have been reported incidentally, or in connection with other chemical studies of sea water, as by Braarud and Klem (1931), Robinson and Wirth (1934), Rakestraw (1932, 1933), Matsudaira and Yasui (1932, 1933), and in connection with

[1] Contribution No. 110.

the Antarctic cruises of the "Discovery" (1932). Three good seasonal studies have appeared more recently, by Soot-Ryen (1932), Verjbinskaya (1932), and Cooper (1933). The last of these is most comprehensive, containing very complete data for the relationship between various chemical factors in the water. Unfortunately, its limitation to one or two shallow stations in the English Channel raises a question as to the validity of its conclusions when applied to other, more general conditions.

The present paper includes results collected over a period of five years at the Woods Hole Oceanographic Institution in the effort to explain the production and utilization of nitrite and its place in the nitrogen cycle of the sea and the general process of marine metabolism. The data include analyses from:

1. A small group of inshore stations in shallow bays.
2. Stations on the continental shelf to the south of Cape Cod, including two serial sections to the edge of the shelf.
3. A number of stations in the Gulf of Maine, so grouped as to indicate seasonal variations for more than a year, at seven different locations.
4. A group of deep sea stations to the south of Cape Cod, from the edge of the continental shelf to Bermuda, at different times of the year.
5. Stations to the south of Bermuda, across the edge of the Sargasso Sea to the Bahamas, and fifteen stations in the Caribbean.
6. A large number of scattered surface samples, at various places and times.
7. Bottom muds and waters contained in them.
8. Samples stored under a variety of conditions.

Methods

The Griess-Ilosvay method, used in this work as in that of practically all previous investigators, has been repeatedly described, with minor variations. It depends upon the color developed in the sample by means of sulphanilic acid and alpha-naphthylamine (or its dimethyl or diethyl derivative). Buch (1923) established its suitability for sea water by a careful spectrophotometric study. It is simple, reliable and not easily susceptible to temperature changes and other fortuitous factors. There is no salt effect upon the color development, so that standards may be made up in distilled water. It was easily established that the color developed was constant between 2 and 48 hours, an adequate range for routine purposes.

Two milliliters of sulphanilic acid solution (8 grams in 1 liter of

30 per cent acetic acid) are added to 100 ml. of the sample, followed by 2 ml. of a solution of alpha-naphthylamine, or diethyl-alpha-naphthylamine (8 grams in 1 liter of 30 per cent acetic acid, with 10 ml. of conc. HCl added). After two hours or more the samples are compared with appropriate standards. This was done in a colorimeter of the Hehner tube type, which has long been used in this laboratory for phosphate, nitrite and other determinations.

We have seldom had trouble in obtaining nitrite-free distilled water (as Cooper seems to have had), and have very frequently found sea water samples free of nitrite to the limit of sensitivity of the method, which is well under .005 microgram atom per liter. Stock standards have been made either from sodium nitrite or from silver nitrite, precipitated by NaCl. In either case, such solutions may be preserved for many weeks and their concentrations checked by titration with $KMnO_4$, or iodimetrically. Dilute standards for use are prepared from these and kept for not longer than one day.

TABLE I

Accuracy of the analytical method for nitrite.

Depth in meters	Triplicate analyses			
	A	B	C	
30	4.3 .307	4.3 .307	4.4 .315	mg./cu. m. µg. at./liter
40	1.9 .135	1.9 .135	1.9 .135	mg./cu. m. µg. at./liter
50	1.6 .115	1.6 .115	1.65 .118	mg./cu. m. µg. at./liter

Throughout this work results will be expressed in microgram atoms per liter, according to the recent recommendations of the International Council. These may be translated into the older units of milligrams of nitrite-nitrogen per cubic meter by multiplying by 14.

In order to indicate the accuracy and reproducibility of results by this method there are given in Table I the results of triplicate analyses (A, B, C) on three different water samples, expressed in both the old and new systems of units. Later results are given to the nearest .005 microgram atom.

Variability of Nitrite

As far as our experience goes, the nitrite concentration in sea water varies normally from 0 to .35 microgram atom per liter. Occasional values up to double this amount have been encountered, and the highest found (in Cape Cod Bay) was .91. No values like those reported by Atkins (2.78, or 38.9 mgm./m.^{3}), or by Matsudaira and Yasui, in the Yellow Sea, have ever been found in normal waters on this side of the Atlantic.

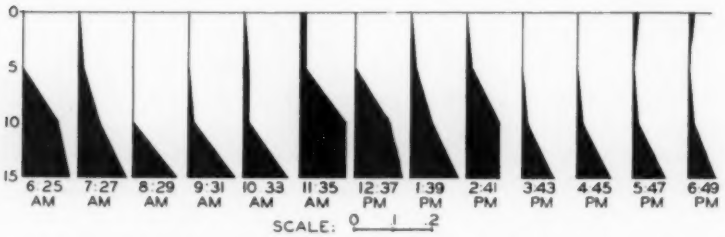

Fig. 1. Variation of nitrite during a tidal cycle. Station K-2, Buzzards Bay. Ordinates, depths in meters.

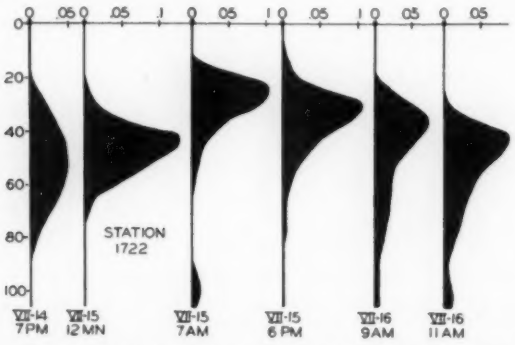

Fig. 2. Variation of nitrite during a tidal cycle. Station 1722, Gulf of Maine. Ordinates, depths in meters.

Nitrite is one of the most variable factors in the sea. Analyses from two stations close together, or from the same station at different times not far apart, sometimes yield quite different results. In fact, the horizontal distribution of nitrite is highly dependent upon local conditions. This would be better appreciated if previous data had not been so scattered and incomplete. Cooper seems to have been the only one sufficiently well aware of the fact.

In view of this, it is rather surprising that any general trends can be seen and conclusions drawn. Absolute values for nitrite concentration, especially isolated ones, are of little use; only by a comparison of relative amounts, with fairly complete data, can one obtain a clear picture of conditions.

To show how general trends do appear, however, the nitrite concentration was followed at two stations (K–2 and 1722) throughout a complete tidal cycle. The results are shown in Figs. 1 and 2. Here, as in all the subsequent figures, the vertical distribution of nitrite is represented by a solid black area.

The vertical variation in nitrite concentration is one of the most striking and interesting facts concerning it. As will later be shown, both the production and utilization of nitrite are processes taking place

TABLE II

Stations in shallow bays. Nitrite concentrations in microgram atoms per liter.

Station:	K-1	K-2	K-3	L-1		L-2		R-3	R-4	S-1	S-2
Depth in meters	8-30 1931	8-30 1931	8-30 1931	7-18 1931	8-28 1931	7-18 1931	8-28 1931	8-26 1932	8-26 1932	7-14 1932	7-14 1932
0	0	0	0	0	0	0	.07	0	0	0	0
5	0					0	.01	0	.41		
10	.01	0	0	0	.01	0	.10	.085		.61	.86
15		.01									
20			.01	0	.01	0	.085		.43		.20
40										.91	

in particular, but different, layers. This often gives rise to large vertical differences, and usually, in the summer, a maximum zone, of variable depth, sharpness and intensity. Good examples of this are found in Atkins' results from the English Channel. Strangely, Cooper's data from the same location, a few years later, fail to show the phenomenon —probably, as he suggests, due to different hydrographic conditions that year.

The nitrite concentration in bottom muds is most highly variable and will receive special attention later.

All this variability in nitrite concentration is only to be expected from the precarious position which it occupies in relation to other substances in the water. Conceivably, it may be produced by oxidation of ammonia or reduction of nitrate, by means of bacteria, catalytically or photochemically. It is not impossible that the phytoplankton may play a part in the oxidation or reduction. In any event, nitrite is one step

in the process of decay and "mineralization" of organic matter, either formed or particulate, or dissolved in the water.

On the other hand, nitrite may be destroyed by: (1) oxidation to nitrate or reduction to ammonia—atmospherically, photochemically, or

TABLE III

Stations on the continental shelf, near Cape Cod. Nitrite concentrations in microgram atoms per liter.

Station:	1270	1295	1271	1296	1272	1297	1273	1298	1260	1299
Depth in meters	6-29 1932	7-20 1932	6-29 1932	7-20 1932	6-29 1932	7-20 1932	6-29 1932	7-21 1932	6-28 1932	7-21 1932
0	0	—	.06	—	.01	.05	.07	—	—	—
10	0	0	0	0	0	0	.01	0	0	.01
20	.01	0	0	0	0	.01	.01	0	0	.01
30		.01		.115		.235		.01		0
40		.085	.07		.14		0			
50								.41		
60						.09			.23	.19
75					0		.26	.36		
100										
125									0	.01
150										
175										.01

Station:	1261	1283	1282	1281	1280	1725	1739	1684	2143	2156
Depth in meters	6-28 1932	7-1 1932	7-1 1932	7-1 1932	7-1 1932	7-25 1933	7-31 1933	7-27 1933	5-8 1934	5-11 1934
0	0	0	0	0	0	0	0	0	.04	0
10		0	0	0	0	0	0	0	.05	0
20	0	0	0		0	0	.02	0	.06	0
30		.17	0	0			.44	.01	.07	0
40		.065				.44	.39	.01		
50			.06	.33	0		.36	.12	.08	.085
60						.20	.24	.03		
75							.36	0	.12	.11
100	.01			.20	.07	.02		0	.18	.27
125								.01	.26	.26
150						.03		.05	.17	.07
175					.05				.05	.08
200	0									.01

by bacteria; or, (2), consumption by phytoplankton. We cannot be altogether certain whether or not free nitrogen enters into the process in any way. However, at any moment, the concentration of nitrite is an expression of the equilibrium between production and destruction.

The distribution of nitrite may therefore be related to a large number

of factors: the distribution of bacteria, phytoplankton and zoöplankton; temperature; light intensity; oxygen tension in the water; density and circulation of the water; and perhaps other seasonal influences.

TABLE IV

Serial sections on the continental shelf. Nitrite concentrations in microgram atoms per liter.

Line "A"
July–August, 1931

Depth in meters	Miles off:									
	1	2	5	7	10	13	39	61	82	90
0	0	0	0	0	0	0	0	0	0	0
10	.07			0	0	0	0	.09	0	
25		.13	.06	.04		.10			0	0
35					.03	.115				
50									0	0
65							.40			
100								.135		0
565									.035	
885										0

Line "D"
August 5–6, 1931

Depth in meters	Miles off:								
	1	2	5	10	20	30	40	50	66
0	0	0	0	0	0	0	0	0	0
10			0						
20	.05	.01		0	.01	0	0	0	0
40			.09	.215					
60					.66				0
75						.05	.15	.165	
100									0
200									.035

DESCRIPTION OF DATA

The results from shallow, inshore stations, shown in Table II, were mostly obtained in the early stages of the work and are useful in demonstrating that the occurrence of nitrite is not closely associated with such influences as land drainage. Although concentrations are usually low, there are exceptions, as at Stations S–1 and S–2, where the highest concentrations of all were found. Great differences in local conditions evidently exist.

The stations on the continental shelf, shown in Tables III and IV, and in Fig. 3, present a fairly regular picture. Since these were all sampled during the summer months, nitrite was seldom found at the surface in more than small traces, while significant amounts were encountered below 30 meters, or near the bottom, if shallower than that. Surface conditions off New York Harbor were somewhat exceptional. Lines *A* and *D*, to the edge of the shelf, bring out the fact that the rate of nitrification on the bottom, as indicated by nitrite concentration there, is apparently greatest at depths of 50 to 75 meters. The number-

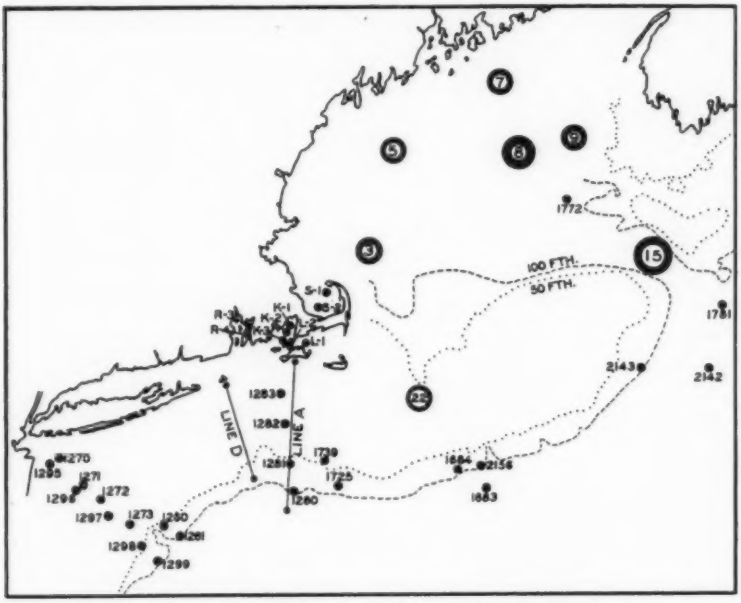

FIG. 3. Location of stations in the Gulf of Maine and vicinity.

ing of stations on these lines (*A*–5, *A*–39, *D*–20, etc.) indicates the number of miles off-shore, in each case. An unfortunate spacing of the sampling levels at the deeper stations on these lines, as well as elsewhere on the shelf, failed to reveal the zone of maximum nitrite generally found at such stations between 30 and 50 meters, and which probably existed here also. At the deepest stations, along the edge of the shelf (1725, 1684, 2143 and 2156), this maximum zone is clearly seen. Its depth varies considerably, showing the influence of the adjacent oceanic water, in which the maximum zone is invariably deeper than at stations on the shelf.

TABLE V

Deep sea stations, Cape Cod to Bermuda. Nitrite concentrations in microgram atoms per liter.

	Group 1			Group 2				Group 3					Group 4		Miscellaneous							
Station:	1731	2313	2455	1732	2315	2456	2457	1462	1734	2316	2458	2460	1465	2317	1463	1640	1683	1730	1781	2142	2450	2451
Depth in meters	7-26 1933	1-21 1935	7-22 1935	7-27 1933	1-21 1935	7-22 1935	7-23 1935	2-7 1933	7-28 1933	1-23 1935	7-23 1935	7-24 1935	2-10 1933	1-23 1935	2-7 1933	5-17 1933	7-27 1933	7-27 1933	9-10 1933	5-7 1934	7-21 1935	7-21 1935
0	0	.02	0	0	.08	0	.01	—	0	.065	0	0	.16	.065	—	.10	0	0	0	0	0	0
10	0		0					.14					.01		.035		0	0	0	0		
20	0	.06	0	0	.15	0	0	.14	0	.065	0	0	0	.06	.01	0	0	0	0	.02	0	0
30	0			0									0		.01	0	0	0	0			
40	0							.15					.03				0	0	0	.12		
50	0	.30	.33	0	0	.215	.30	.135	0	.06	0	0	0	.03	.01	0	.08	0	.18		0	0
60	0			0				.15	0								.06	.03	.165	.38		
75	.20	.06		0	.16				.01	.05	0	0		0	.03		.01	.02	.05	.60		
100	.065	.04	0	0	.32	.14	.06	.21	.05	.06	0	0		0	.03	.06	.01	.02	.02	.51	0	0
150	.01	0		.05	.14			.185	.02	.065	0	0	0	.065	.035	.07	.02	.01	.02	.29	0	0
200	.01	0	0	0	.08	0	0	.185		.06	0	0	.03	.035	.01	.04	.03	.01	.02		0	0
250		0			.08				0	.065		0					.03		0	.01	0	0
300		0	0	.065	0	0	0	.01	0	.065	0	0	0	.06	0	.04	.02		0	0	0	0
400	.01		0	.01	0	0	0	.165	0	0	0	0	0	0	0	.01	.02	.01	.01	.01	0	.01
500			0			0	0	.07			0	0	.02	0	0	0	0		.01	0	0	0
600	0		0	.01	0			.01	.02	0	0	0	.03	0	0	0	0	.01	0	.02	0	0
800	0			0	0			.01	0	0	0	0	0		0	0	0	.06	0	0	0	0
1000	0			0	0			0		0	0	0	0		0	0	0	.01	.01	0	0	0
1100															0		0					
1200	0				0			.01			0	0	0		.01		0	0	.01		0	0
1400	0							0					0		0		0	0	.02			
1600	0							0					0		0			0	.02		0	0
1800													0						.01	0		
2000								0					0		0			0	.03	.02	0	0
2500													0		0						.035	.01
3000													0		.035							
3500															.04							
4000															0		0					

TABLE VI

Seasonal variation of nitrite in the Gulf of Maine. Nitrite concentrations in microgram atoms per liter.

Location 3

Station:	1284	XX	1699	1748	1857	1864	1909	2022	2074	2168	2220	2274
Depth in meters	7-12 1932	5-6 1933	7-3 1933	9-4 1933	10-19 1933	12-2 1933	1-9 1934	3-22 1934	4-18 1934	5-22 1934	6-26 1934	9-19 1934
0	0	0	0	0	.215	.09	.08	.085	.10	.01	.035	0
10	0	0	0	0		.08	.02	.085	.10	0		0
20	0	.06	0	0		.08			.115	0	.02	
30	0	.17	.01	0	.28	.08	.03	.11	.135	0	0	.11
40	.165		.14	.04						.22	.18	
50	.32	.20	.11		.21	.07	0	.085	.135		.135	0
60	.32		.07	.02		.065				.39	.09	0
80			.01	0	0	0	0	.09	.06	.14	.04	0
100	.02	.02	0	.03	.01	0	0	.085	.02	.115	.02	0
125			0	0				.065	0	.10	.02	0
150	0		.01	0	.01	0	.01	.04	0	.01	.03	0
175								.03	0	0	.01	0
200	.035		0	0	0	0	0		0	0	.02	0
225		0						.03	0	0		
250			0	0	0		0					0

Location 5

Station:	1287	1705	1761	1871	1911	2031	2083	2176	2224	2282
Depth in meters	7-10 1932	7-7 1933	9-6 1933	12-3 1933	1-9 1934	3-23 1934	4-19 1934	5-24 1934	6-28 1934	9-20 1934
0	.035	0	0	.035	0	.09	.11	.08	0	0
10		0	0	.035	0	.085	.115	0	0	0
20	0	0	.08	.02	0		.12	.18	0	
30	.10	.07	0	.02	0	.085	.115	.21	.28	.035
40	.25	.085	0	.02	0			.21	.235	
50	.09	.09	0	.02	0	.08	.12	.215	.215	0
60	.03	.04	0	.03	0			.185	.15	
80	.01	.01	0	.02	0	.05	.05	.115	.15	0
100	.01	.01	0	.01	0	.035	.035	.085	0	0
125	.01	.01	0	.01	0	.02	.03	.10	0	.01
150	.03	.01	0	.03	0	.03	.01	.05	0	0
175		.01	0		0	.035	0	0	0	0
200					0				0	

Table V contains the deep stations from Cape Cod to Bermuda. A number of these are located in roughly corresponding positions and are arranged in four groups accordingly. Of these, No. 3 is most complete, with four stations at different times of the year. These have been plotted in Fig. 8 to show the seasonal variations. At all these stations, the maximum zone is found between 50 and 100 meters, with only traces

TABLE VII

Seasonal variation in the Gulf of Maine (continued). Nitrite concentrations in microgram atoms per liter.

Location 7

Station:	1712	1765	1888	1916	2037	2089	2182	2286
Depth in meters	7-8 1933	9-7 1933	12-7 1933	1-10 1934	3-24 1934	4-20 1934	5-25 1934	9-21 1934
0	0	0	.03	.02	.065	.09	.12	.19
10	0	.01	.02	.01	.07	.10	.12	.35
20	.165	.16	.03	.01			.13	
30	.22	.215	.05	.01	.065	.10	.11	.33
40	.21	.16	.035				.11	
50	.18	.04	.05	0	.06	.12	.06	.31
60	.17	.035	.035				.06	
80	.17	.035	.03	0	.06	.13	.09	.30
100	.065	.035	.02	0	.065	.13	.10	.06
125	.02	0		.02	.06	.07	.085	0
150	.03	.01	.02	.02	.04		.08	0
175	.04	.02					.05	0
200			0					0

Location 8

Station:	1716	1764	1853	1886	1914	2035	2087	2180	2227	2288
Depth in meters	7-9 1933	9-7 1933	10-19 1933	12-6 1933	1-10 1934	3-24 1934	4-20 1934	5-25 1934	6-29 1934	9-21 1934
0	0	0	.32	.085	.06	.065	.04	.065	0	0
10	0	0		.08	.06	.05	.05	.01	0	0
20	0	0	.28	.085			.11	.03	.24	
30	.115	.07		.07	.05	.06	.115	.11	.085	.10
40	.21	.01	.40	.08				.08	.07	
50	.215	0		.085	.03	.05	.115	.165	.05	.04
60	.12	.01	.15	.085				.17	.05	
80	.02	0	.16	.07	.01	.06	.08	.17	0	0
100	0	.02	.08	.085	.01	.01	.035	.15	0	0
125	0	.02				.01	.03	.135	0	.035
150	.04	.01	.01	0	0	0	0	.13	0	.01
175	.02	0			0	0	0	.09	0	0
200		0	.08	0		0	0	.05	0	.01
225					0	0	0	0		.04
250		0			0					0

of nitrite below this. Only during the winter months is nitrite found at the surface. The extremely sharp maximum zone at Station 1781 may be noted, at which nearly all the nitrite in the water column occurs in a twenty-meter layer, somewhat nearer the surface than at stations further to the south.

Stations in the Gulf of Maine, shown in Tables VI, VII and VIII

are grouped into seven "locations." These include Location 22 on Georges Bank, which may be considered an adjunct to the Gulf of Maine. The separate stations in each of the locations are close enough

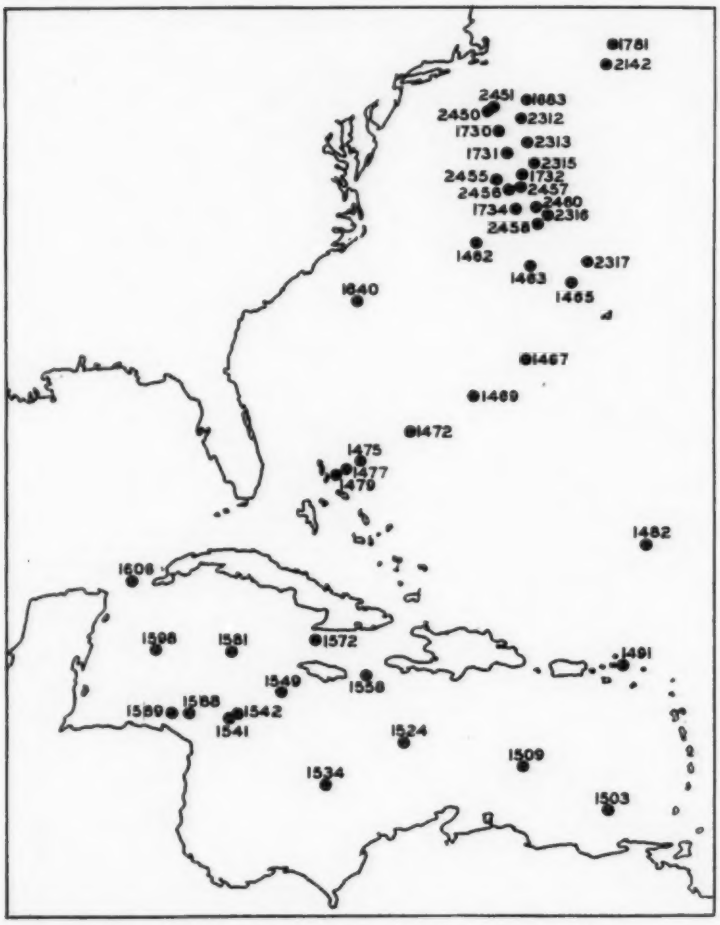

FIG. 4. Location of stations from Cape Cod to the Caribbean.

together to be comparable, and were visited on different cruises at various times of the year, covering a total period of thirteen months or more. The data from six of these locations have been plotted in Figs. 5, 6, and 7 to show the seasonal variations of both nitrite and nitrate. The

data from Location 9 (not tabulated) are shown in Fig. 12, to indicate the relation between nitrite concentration, temperature and density.

These figures from the Gulf of Maine are but a small portion of the

TABLE VIII

Seasonal variation in the Gulf of Maine (continued). Nitrite concentration in microgram atoms per liter.

Location 15

Station:	1659	1781	1846	1899	1924	2052	2118	2137	2197	2296
Depth in meters	6-22 1933	9-10 1933	10-19 1933	12-7 1933	1-11 1934	3-26 1934	4-30 1934	5-6 1934	5-28 1934	9-23 1934
0	0	0	.19	.14	.11	.085	0	0	.01	0
10	0	0		.135			0	0	0	0
20	0	0	.14	.15	.065	.085	.07	0	0	
30	.01	0		.15			.11	0	0	0
40	.02	0	.19	.085	.06	.10			0	
50	.06	.18		.08			.115	.11	.065	.01
60	.09	.165	.11	.035	.06	.10			.115	.11
80	.05	.02	.01	0	.01	.10	.08	.16	.185	.265
100	.05	.02	.02	0	0	.07	.16		.085	.07
125	.01	.02				.01	.135	.04	.01	
150	.01	.02		0	0	0	.13	.05	.03	.035
175	.01	.01				0	.03	.03	.115	.02
200	.01	.02	.02	0	0	.07		.01	.115	.01
225						.06			0	
250	.01	0	.02	0	0	0		0		0

Location 22

Station:	1686	1742	1934	2069	2154	2214	2269
Depth in meters	7-27 1933	9-3 1933	1-13 1934	3-29 1934	5-10 1934	6-2 1934	9-18 1934
0	0	0	.03	—	.05	.05	.04
10	0	0		.035	.04	.06	.06
20	.03	.085	.035		.035	.04	
30	.035	.15		.03	.035	.05	.08
40	.04	.165	.03			.05	
50	.04	.18		.07	.03	.05	.15
60	.04	.185	.03	.035		.05	
80		.185				0	.21

analyses made during a survey of that region, the results of which will appear at a later time.

The stations to the south of Bermuda, including those in the Caribbean (Table IX), present a rather different picture from that in the north. Generally, smaller amounts of nitrite are found, but frequently

relatively large amounts at the surface. The maximum zone is near 100 meters, sometimes sharp, sometimes poorly defined.

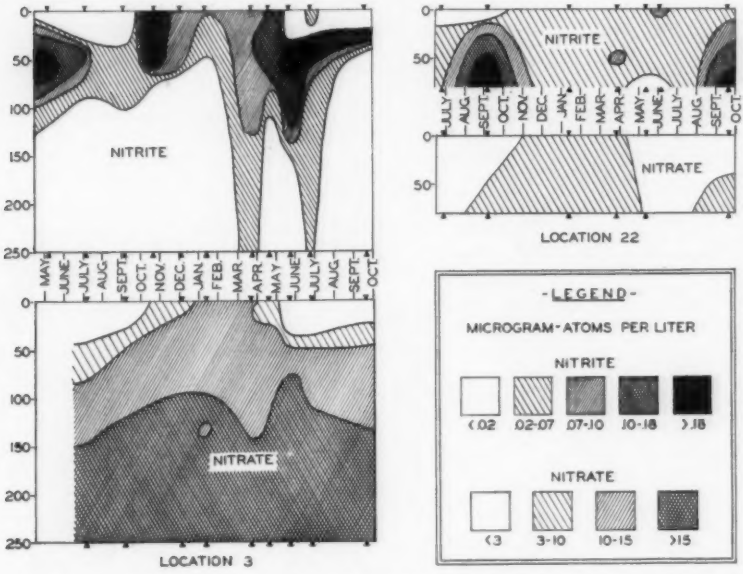

FIG. 5. Seasonal variation of nitrite and nitrate. Locations 3 and 22, Gulf of Maine. Ordinates, depths in meters.

RELATION OF NITRITE TO OTHER FACTORS

Nitrate and Phosphate

Since nitrite participates in the metabolic cycle with the other "nutrient salts," it would seem important to compare its variations and behavior with that of the other nutrients, nitrate and phosphate.

Figures 9 and 10 show these relations at a number of stations in the Gulf of Maine at various times of the year. Certain conclusions are evident:

1. High nitrite concentrations in the upper water levels are associated with high concentrations of nitrate and phosphate. The converse is not always true, especially at certain times of the year. During the winter, when the surface is being replenished with nutrients by overturn of the water mass, nitrite is also in evidence, particularly as the season advances. In January, the production of nitrite is at a minimum, and since this corresponds to the time of greatest instability of the water mass it occasionally happens that little or no nitrite is found at any level,

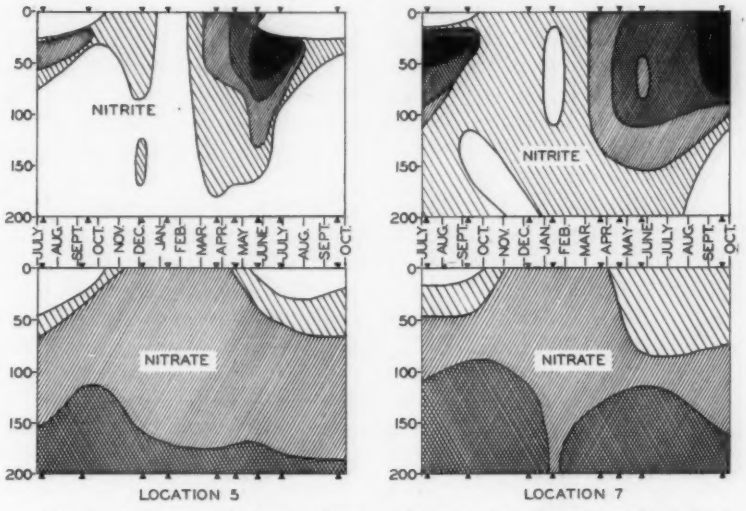

FIG. 6. Seasonal variation of nitrite and nitrate. Locations 5 and 7, Gulf of Maine. (See Fig. 5 for legend.) Ordinates, depths in meters.

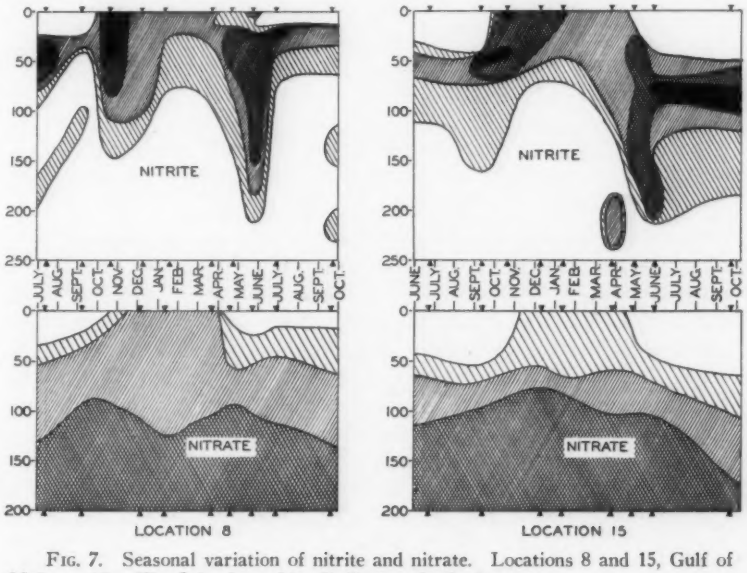

FIG. 7. Seasonal variation of nitrite and nitrate. Locations 8 and 15, Gulf of Maine. (See Fig. 5 for legend.) Ordinates, depths in meters.

even though there is abundant nitrate. This condition obtains at Stations 1908 and 1910.

2. High nitrate and phosphate in the *deeper* water is not associated with nitrite. The latter is seldom found in large amounts below 100 meters. It is evidently produced near the surface and works down through the unstable layer during the winter until stratification again sets in.

3. When diatom growth begins in the spring, with the utilization of nitrate and phosphate, nitrite also disappears in the surface water. At Station 2099 this process has apparently just begun, while at 2098 the season is further advanced, nitrate and phosphate are down almost to their summer values at the surface, and nitrite is absent. Figure 10 is compiled from a number of stations during the summer months to

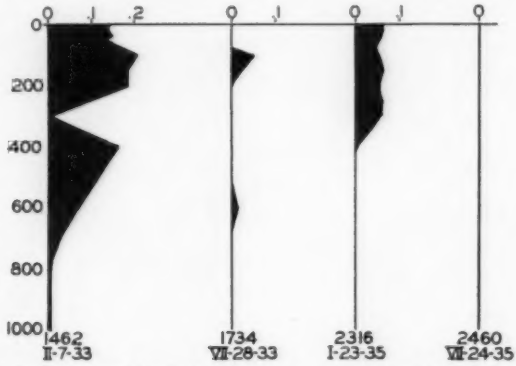

FIG. 8. Seasonal variation of nitrite at an oceanic station. (Group 3, Table V.) Ordinates, depths in meters.

show the close correlation between the gradients of nitrite, nitrate and phosphate near the surface. The origin points for nitrite and nitrate coincide in these diagrams, but the phosphate curves are arbitrarily displaced to the left to facilitate comparison. The shape, slope and position of the curves indicate that the same conditions govern all three. As a consequence of this correlation, it is proposed that nitrite, which is normally produced near the surface, is utilized by the phytoplankton during the period of its growth, in the same manner as nitrate and phosphate, so that the upper levels become depleted. If the upper right-hand corner of the nitrite area shown in Fig. 9 for Station 1896 (or 1884) were cut off, and the curves for nitrate and phosphate deflected towards the origin, a condition would be represented exactly similar to those in Fig. 10. This is what would be expected during July or September. Abundant evidence of the utilization of nitrite by diatoms is

TABLE IX

Stations south of Bermuda, including the Caribbean. Nitrite concentrations in microgram atoms per liter.

Station:	1467	1469	1472	1475	1477	1478	1482	1491	1503	1509	1524	1534	1541	1542	1549	1558	1572	1581	1588	1589	1598	1606
Depth in meters	2-14 1933	2-15 1933	2-17 1933	2-19 1933	2-19 1933	2-20 1933	3-3 1933	3-8 1933	3-15 1933	3-23 1933	3-29 1933	4-2 1933	4-4 1933	4-4 1933	4-5 1933	4-15 1933	4-25 1933	4-27 1933	4-29 1933	4-30 1933	5-1 1933	5-4 1933
0	0	.06	0	0	0	.01	.05	.07	.24	.35	.06	0	0	.04	.265	.04	.10	.165	0	.04	.01	.135
25	0	0	0	0	0	0	.01	.03	.14	.02	0	.02	0	.01		.02		0	0	0	0	.01
40	0	0	0	0	0	.02																
50					0		.05	.01	.18	.01	0	.01		0	.01	.01	.05	0	0	0	0	.01
100	0	.07	0	0	0	.035	0	.01	.05	0	0	.06		.32	.03	.02	.035	.185		.47	0	.11
150	0	.03	0	0	.11	.04	.03	.02	.035	.06	.035	.03		.02	.035	.04	.16	.035		0	.02	.035
200		.03			.01	.035	.035	.01	.01	.02	.02	.03		.02	.02	.02	.035	0		0	.01	.03
300	0	0	0	0	0	.01	.035	.09	.01	.01	0	.01			.02	.03	.03	0		0	0	.02
400	0	0	0	0	0	.01	.02	.02	.02	.01	0	.03			.01	.02	.02	0		0	0	.01
500		.01	0	0	0	.01	.02	.01	0	.01	0	.01			.02	.03	.01	0		0	.01	.01
600	.01	0	0	0	0		0	.04	.01	0	0	.02			.01	.02	.02	0		0	0	.01
700							.02	.04	.02											0		
800	0	0	0	0	.01		.01			0	0	.01			.01	.02	0				0	.01
900		0			.01		.01	.035		0												
1000	0	0	0	0	0		0	.01			0	.01			0	.02	.01					
1100							.03	.01														
1200	0	0	0	0	.01			.02			0											
1400	0	0	0	0	0		0	.02			0						.01					
1600	0	0	0	.01	.01		0	.02														
1800	0	0						.04														
2000	0	0	0	0	0		0				0						0					
2500			0	0	0		0				0											
3000	0	0		0	0		0				.01						.01					
3500											0											
4000	0	0									.01						.01					

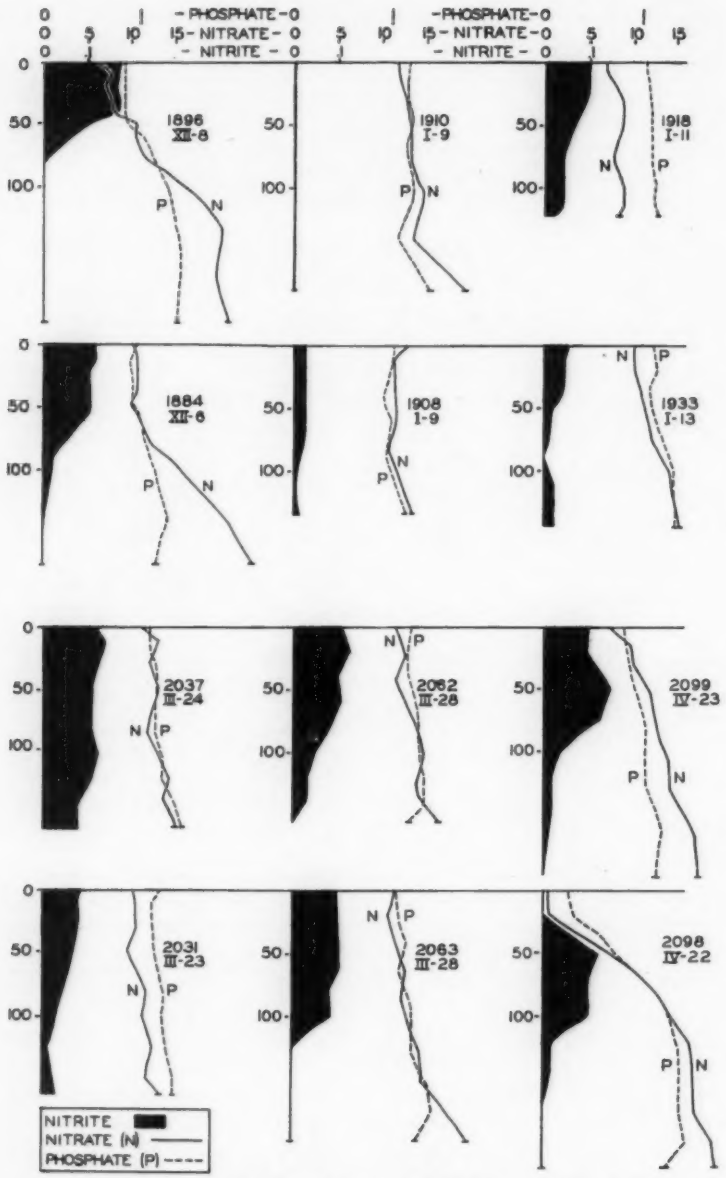

FIG. 9. Relation of nitrite to nitrate and phosphate. Various stations in the Gulf of Maine at different times of year. Ordinates, depths in meters. Positions of stations as follows:

Station	Position	Station	Position	Station	Position
1896	42°48′N 67°15′W	1910	42°43′N 69°15′W	1918	43°39′N 66°39′W
1884	43°00′N 68°37′W	1908	42°10′N 69°53′W	1933	41°25′N 69°02′W
2037	43°58′N 68°03′W	2062	42°44′N 68°44′W	2099	42°22′N 68°32′W
2031	43°18′N 69°23′W	2063	42°31′N 68°31′W	2098	42°50′N 67°19′W

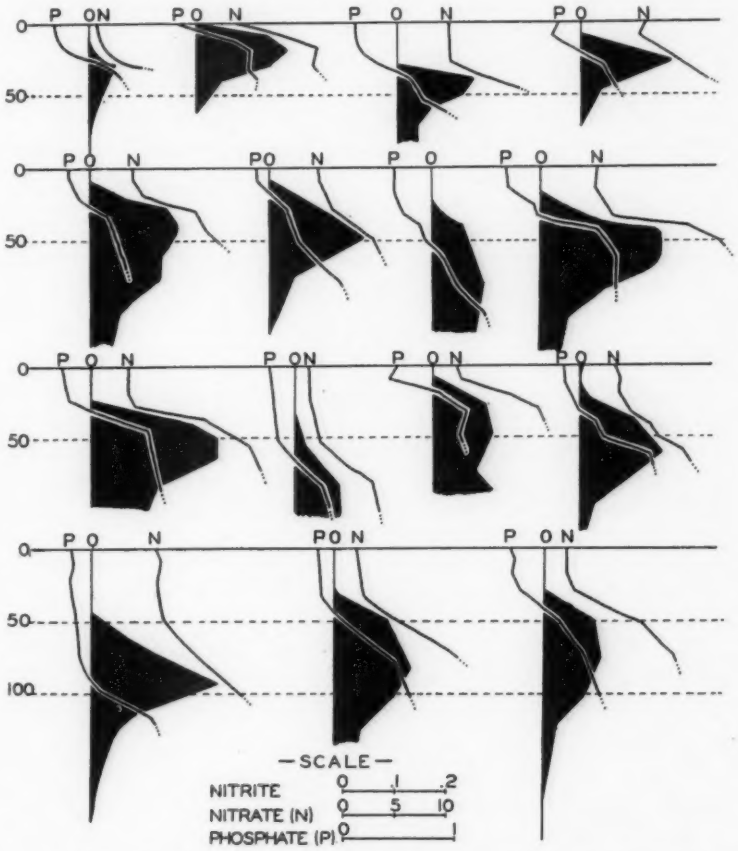

FIG. 10. Relation of nitrite to nitrate and phosphate. Various stations in the Gulf of Maine in the summer. (The origins for nitrite and nitrate coincide, but the phosphate curves are arbitrarily displaced to the left, to facilitate comparison of all three.) Ordinates, depths in meters.

afforded by Schreiber (1927), Cooper (1933), ZoBell (1935) and others.

But the Gulf of Maine is a rather specialized area; let us turn to the open ocean. Similar relations between nitrite, nitrate and phosphate are shown in Fig. 11 for a number of deep sea stations from Latitude 12 to 42 N. Most of these show a clearly-defined nitrite maximum, at about 100 meters, but all show nitrite at the surface, except the three northern stations, which were visited during the summer. At most of them there is a small but distinct amount of nitrite below 150–200 meters. Two stations, 1482 and 1491, are somewhat exceptional. At these, nitrite concentrations are rather low, but extend further down, with no well-defined maximum. The fertility of a region is obviously concerned in nitrite production and these stations are on the edge of the barren Sargasso Sea. Other stations in the Sargasso, as shown in Table IX, are largely devoid of nitrite.

Nearly all the nitrite formation takes place well above the level of maximum nitrate and phosphate concentration, which is found at about 800 meters south of the Gulf Stream and 400 meters north of it.

Density and Temperature

Figure 12 shows the relation between nitrite, density and temperature at stations in Location 9 at various times of year. It can readily be seen that:

1. As the inflection point in the density curve moves further down nitrite moves down also. This is another clear evidence that nitrite is formed near the surface and diffuses or mixes downward, more completely the more unstable the water column.

2. The production of nitrite goes on even at low winter temperatures, but with the rise in temperature nitrite is utilized as diatoms begin to grow.

3. The occurrence of a sharp nitrite maximum coincides with a high density gradient. If the depletion of nitrite above this is the result of diatom activity, this is localized and intensified by strong stratification.

Ammonia

Unfortunately, it was not possible to include simultaneous determinations of ammonia with those of nitrite and nitrate throughout this study. A few such determinations were made, however, using the method described by Krogh (1934). The results are shown in Table X, correlated with nitrite and nitrate. The low values for all three at the surface of Station 1739, and their increase toward the bottom, lend support to the view that they are all utilized at the surface and that

ammonia is oxidized to nitrite and nitrate below. The increase of ammonia at the surface of Station 1742 (in Location 22) in September would seem to indicate that organic decomposition is going on more rapidly at this time, and ammonia being liberated, but that the second

TABLE X

Relation of nitrite to ammonia and nitrate. Concentrations in microgram atoms per liter.

Station	Depth in meters	Nitrite	Ammonia	Nitrate
	77	.01	.4	1.1
1734	340	0	.3	4.6
	855	0	.3	19.5
7/28/33	2650	(0)	.6	20
	3280	(0)	.4	
	3725	(0)	.3	20.5
	4180	(0)	.6	23
	0	0	1.2	1.1
	10	0		1.1
1739	20	.02	1.4	2.5
	30	.44		4.0
7/31/33	40	.39	4.3	5.7
	50	.36		5.7
	60	.24	4.1	7.1
	75	.36	4.3	6.4
	0	0	—	1.1
	10	0	2.8	0
1742	20	.085	3.0	2.1
	30	.15	3.2	4.3
9/3/33	40	.165	3.2	6.1
	50	.18	3.8	7.5
	60	.185	4.2	7.1
	75	.185	—	7.5
2312	15	.04	.8	>13
	200	.05	0	10
2313	15	.02	.8	6.4
2314	15	.05	.7	2.8
2315	5	.08	.7	1.4
2316	5	.065	0	1.8
2317	5	.065	.3	1.7

stage—appearance of nitrite—has not yet been reached. Or it may be that late in the summer, although nitrite is still being utilized, the ammonia is not drawn upon as a nutrient source. This was Cooper's assumption on finding the same condition in the English Channel.

At the various depths at Station 1734 the ammonia and nitrite are practically constant throughout, but the nitrite varies widely. From Station 2312 to 2317 the nitrate progressively diminishes, but both nitrite and ammonia are approximately constant. In fact, throughout all the data shown the nitrite is more closely related to ammonia than to nitrate.

Unpublished data in the hands of Dr. A. C. Redfield, on the distribution of ammonia in the Gulf of Maine in May and June, show a distinct tendency for ammonia to concentrate between 20 and 50 meters in a maximum zone similar to that for nitrite.

Nitrite Changes in Stored Samples

A number of previous investigators have tried to determine what happens to the nitrite in samples on standing. The results are altogether irregular and confusing. Buch (1923) reported the formation of as much as 28 mg./cu.m. of nitrite-N in two days, in samples standing in the dark. Harvey noted the appearance of nitrite in a few cases. Orr found increases in nitrite in diatom cultures, even after the diatoms had stopped growing. Braarud and Klem cite some experiments on nitrate reduction, but are not specific as to the relation of nitrite. Cooper followed 44 samples for 10–13 days, to some of which nitrite and ammonia had been added. He reports: "In all cases the changes in nitrite were small, little more than the experimental error." Keys, Christensen and Krogh (1935) followed the ammonia (and in a few cases nitrite, nitrate and oxygen) in samples stored for several days. Results were so variable that they finally conclude: "It appears to us that the only conclusion that can be drawn with regard to nitrogen metabolism in stored sea water is that there are usually a number of forces at work, some operating to reduce the free ammonia, others tending to release ammonia; the observed ammonia will always be a resultant of these forces. . . ."

Although with little hope of clearing up this confusion, a number of similar determinations on stored samples were made. The first of these were simply repeated analyses of the ordinary routine station samples made after a number of days standing. No particular precautions were taken to keep the samples in the dark, but they were exposed to probably less than normal illumination. Nearly 300 samples were followed in this way, with the results shown in Table XI.

One section of the table concerns samples which were originally "nitrite-free," or in which the initial concentration was not greater than .01. These are separated into three periods of standing, approximately 1, 2 and 3 weeks. Those which showed a significant increase in nitrite

in that time (greater than .035 microgram atom per liter) amounted to 11 per cent, 12 per cent and 33 per cent of the totals. These 162 samples include many from the surface, with low nitrate, and many from deep levels, with high nitrate. There was no difference in the behavior of these two classes, a fact which has a bearing on the question of reduction of nitrate to nitrite.

The other group of 178 samples contained initial concentrations of nitrite from .01 to .91. Changes took place in both directions. But relatively fewer cases of increase, and more of decrease, were found

TABLE XI

Nitrate changes in stored samples

A. Samples with initial concentration = 0 (<.01 microgram atoms per liter)

	5–7 days	12–15 days	20–24 days
Total number	109	41	12
No. increased >.035 microgram atom per liter	12	5	4
Percentage of total	11%	12%	33%

B. Samples with initial concentration >0 (.01–.91 microgram atom per liter)

	5–7 days	12–15 days	20–24 days
Total number	119	25	34
No change (Increase or decrease <10 per cent of initial concentration)	49	9	9
Number increased, over initial concentration, 10–50 per cent	18	1	1
Number increased, over initial concentration, >50 per cent	11	1	0
Number decreased, of initial concentration, 10–50 per cent	31	14	17
Number decreased, of initial concentration, >50 per cent	10	0	7
Percentage of total number which *increased* more than 10 per cent of initial concentration	24%	8%	3%
Percentage of total number which *decreased* more than 10 per cent of initial concentration	34%	56%	70%

as the time of standing lengthened. The predominating tendency under these conditions is for nitrite, if initially present, to disappear on standing. If initially absent, it may appear in small amounts.

The results of these experiments do not lead to a conclusion much more definite than that of Keys, Christensen and Krogh.

Subsequently, however, several series of more carefully controlled experiments were carried out. Samples of filtered surface water were placed in Pyrex flasks. Some of these contained added amounts of ammonia-nitrogen, others added nitrate. Half the samples were steri-

lized in an autoclave, and of the total number, half were stored in the dark and half were placed in the sunlight as much as possible during the period of storage. Analyses for nitrite were made at intervals up to 19 days. Two series of experiments of this sort were carried out, with the following results:

1. The samples stored in the dark, whether sterile or not, showed no significant change in nitrite, even in the presence of added nitrate or ammonia.

2. Those kept in the light invariably showed a change in nitrite. If it was high at the beginning it decreased, but not so sharply in the presence of excess nitrate. If the nitrite concentration was low at the start it increased slightly, but *more* so in the presence of added nitrate. In fact, after 19 days the nitrite reached the same value, whether by increase or decrease. This seems to depend, however, upon the presence of sufficient nitrate, and is independent of the concentration of ammonia. The conditions surrounding the attainment of this apparent "steady state" will be the subject of further investigation.

3. The rate of disappearance of nitrite was unaffected by the presence of excess ammonia, and only a very slight increase was found in samples of low original nitrite content. In view of the conditions of these experiments this result is not necessarily in conflict with those of ZoBell (1933), who reported the photochemical oxidation of ammonia to nitrite under certain conditions.

4. An effort to follow the changes in nitrate led to inconclusive results. In one series of experiments the sum of nitrite and nitrate (determined by Harvey's "nitrate" method, using reduced strychnine as a color reagent) remained constant, although the concentration of nitrite alone decreased considerably; but in another series the increase in nitrate did not compensate for the disappearance of nitrite.

Thiele (1907) was apparently the first to show that nitrate can be reduced to nitrite by ultraviolet light. Moore (1918) found that sunlight can accomplish the same result, but that the shorter wave-lengths are the most active. More recently, Villars (1927) has studied the photochemical decomposition of KNO_3 in considerable detail and has shown that wave-lengths less than 3,000 Ångstroms are effective in the reduction. About 1 per cent of the total energy of the solar spectrum at sea level falls within this range.

Nitrite in the Sea Bottom

It is evident that at shallow stations, at least, a great part of the decomposition of organic matter takes place on the bottom. A study of the nitrite content of bottom sediments might therefore be significant

Brandt gives figures for the ammonia and albuminoid-nitrogen content of "bottom water" (that in contact with the bottom surface) and of "interstitial water" (that contained in the bottom cores brought up) showing from three to five times as much in the latter as in the former. This relation was apparently independent of depth, for the bottom samples varied from 45 to 3,000 meters. He also cites determinations on muds from the North Sea showing 2–4 mg. of ammonia-N and 5–10 mg. of nitrate-N per kilogram of moist mud.

A number of bottom samples were obtained from various sources and determinations made of the nitrite and nitrate in the interstitial and adjacent water. The results are contained in Table XII.

Figures are given for the nitrite and nitrate not only in the bottom samples, but also in the lowest water bottle, which will vary from 2 to 20 meters from the bottom.

Care must be taken in comparing the results, for different methods were used in obtaining and treating the samples. In some cases, the interstitial water was removed from the whole mud core by suction and the nitrite concentration expressed in terms of the volume of water thus recovered. In other cases, samples of mud and the water in contact with it were collected, either in the coring device, the Pettersson grab, or a special mechanism which sucks up a sample of mud and water off the bottom. Nitrite was determined in this water, after filtration.

Although the data from deep stations are fragmentary they are sufficient to show that conditions in deep bottoms are different from those in shallow ones. The single determination at Station 1731 is not quantitative, but it at least shows that there is very little nitrite in the mud, compared with Stations 1331, 2220 and 2227, where similar technic was used. The condition of the sample from Station 1734 is doubtful, but it should approximate those from A-8 and A-25. Nevertheless, the content of nitrite is very low, and the nitrate is very little greater than that in the lowest water-bottle.

The results from Stations 2220, 2224 and 2227 are interesting in showing the enormous quantities of nitrite in the interstitial water of shallow bottoms.

A comparison of these quantities with those in the supernatant "bottom water" shows that the rate of nitrite formation in the mud is very much higher than its rate of diffusion into the surrounding water.

At Station A-8 and again at A-25 two samples were obtained as close together as possible. The variation in their nitrite and nitrate content is partly due to the method of sampling, which affords a mixed sample of mud and water sucked off the bottom. Nitrite was determined in such a sample after filtration, and although this contains "in-

TABLE XII

Nitrite and nitrate in the sea bottom. Concentration in microgram atoms per liter.

Station	Depth in meters	In lowest water-bottle		In bottom		Method of obtaining sample for analysis
		Nitrate	Nitrite	Nitrate	Nitrite	
1734	4630	20.5[2]	0?	23.0[1]	.05	Water-bottle closed on bottom; brought up mud and water.
1731	3840				.04	Portion of core extracted with surface water.
1336	65	5.0	.28	25.0	1.8	Water brought up with sand in Pettersson grab.
1331	200		.115		.63	Portion of core extracted with surface water.
2220	218		.04		.065	Supernatant "bottom water" on top of core.
2220	218		.04		28.3[3]	Interstitial water removed from core by suction.
2224	195		0		.035	Supernatant "bottom water" on top of core.
2224	195		0		3.3	Interstitial water removed from core by suction.
2227	210		.01		.035	Supernatant "bottom water" on top of core.
2227	210		.01		10.8[4]	Interstitial water removed from core by suction.
A-8*	32	A B	.235 .235	4.6 10.3	.26 .74	The sampling device sucks up a sample of mud from the surface of the bottom, along with water in and around it. Contains interstitial and bottom water in varying proportions.
A-25*	45	A 10.3 B 10.3	.13 .13	17.2 24.6	.20 .25	
Near K-3†	15	A B C			.065 .12 .285	
Near K-3*	15	A B			.75 .77	

* Two samples, A and B, taken as close together as possible.
† Three samples, A, B, C, taken as close together as possible.
[1] Ammonia-nitrogen concentration, 0.65.
[2] Ammonia-nitrogen concentration, 0.35.
[3] Total extractable nitrite-N, 1.5 mgm. per kgm. of dry mud.
[4] Total extractable nitrite-N, .25 mgm. per kgm. of dry mud.

terstitial" and "bottom" water in varying proportions the irregularity of results would nevertheless seem to indicate great local differences in bottom conditions.

Similarly, near Station K-3, three samples were taken simultaneously at one place and two at another. The first three were quite different in nitrite content, while the latter two were practically identical.

The general conclusion to be drawn is that the decomposition of organic matter on the sea bottom involves oxidation of nitrogen compounds to nitrite and nitrate, and that this process is much more intense on shallow bottoms than deep. Nearly all of the nitrification must have taken place before organic matter reached the bottom in the latter cases.

The samples from near Station K-3 were analyzed again after having stood for one day. The nitrite in three had approximately doubled; in the fourth it had increased 50 per cent. The water from the fifth mud sample had been separated from the mud at the time of the first analysis, and the nitrite in this water had *decreased* 50 per cent after one day. Evidently nitrite is formed rapidly and continuously in the mud, diffusing into the water in contact with it. In the water, however, the nitrite is rapidly destroyed, presumably by oxidation to nitrate.

Samples of white coral mud from Castle Harbor, Bermuda, at a depth of about 5 meters, were extracted by distilled water and surface sea water, respectively. The distilled water extract contained: nitrate, .85; nitrite, 0. The surface water extract contained: nitrate, 15; nitrite, .085. The surface water itself was nitrite-free and contained .7 microgram atom per liter of nitrate-N. The more efficient extraction by sea water shows the same phenomenon of "base-exchange" so common in the extraction of soils.

Nitrite at the Surface

The question of the occurrence of nitrite in the immediate surface layer seems to merit particular consideration.

The first intimation of irregularity occurred on the section from Cape Hatteras to Bermuda (see Fig. 4). At Stations 1462 and 1463 suspiciously high surface values were obtained. These were traced, apparently, to contamination, and were finally rejected. But later, at Station 1465, there could be no doubt of a fairly high concentration at the surface, with very little immediately below. However, no surface nitrite could be found in the vicinity of Bermuda or at the immediately following stations, except 1469. Between this station and 1472 surface samples were picked up every hour or two, while the ship was in motion, without finding more than a trace of nitrite. The same was true of a number of samples taken up between Stations 1473 and

1475. Much later, six samples taken in the same way between Stations 1730 and 1731 were nitrite-free.

Throughout the Caribbean cruise nitrite was found at the surface at the majority of the regular stations, but at four places where surface samples alone were taken none was found. In all, out of thirty surface samples taken with the ship in motion, detectable traces of nitrite

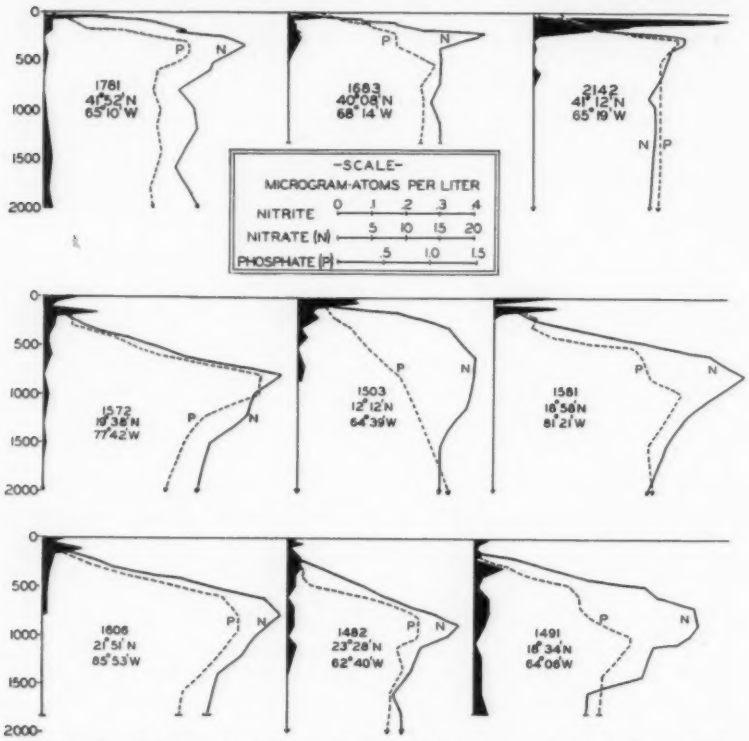

FIG. 11. Relation of nitrite to nitrate and phosphate, at deep stations. Ordinates, depths in meters.

were present in only six, and only once a concentration of more than .01. Whether the occurrence of considerable amounts of surface nitrite in the Caribbean is constant or limited to a certain time of the year (as in the north) cannot be said from the results of this one cruise, but the strange irregularity suggests that special factors—perhaps photochemical—may operate in the immediate surface, a matter which will later be investigated more fully. Highly irregular variations in sur-

face nitrite have also been reported by Matsudaira and Yasui between Nagasaki and Shanghai.

The vicinity of Bermuda is an area especially devoid of nitrite and other "nutrients." A number of surface samples were collected from the harbors and bays of the Bermuda Islands, and even from their fresh water streams and ponds, with no more than traces of nitrite, and minimal amounts of nitrate.

Seasonal Changes in Nitrite

Figures 5, 6, and 7 contain the data from Locations 3, 5, 7, 8, 15 and 22 in the Gulf of Maine, arranged to show seasonal variations of nitrite

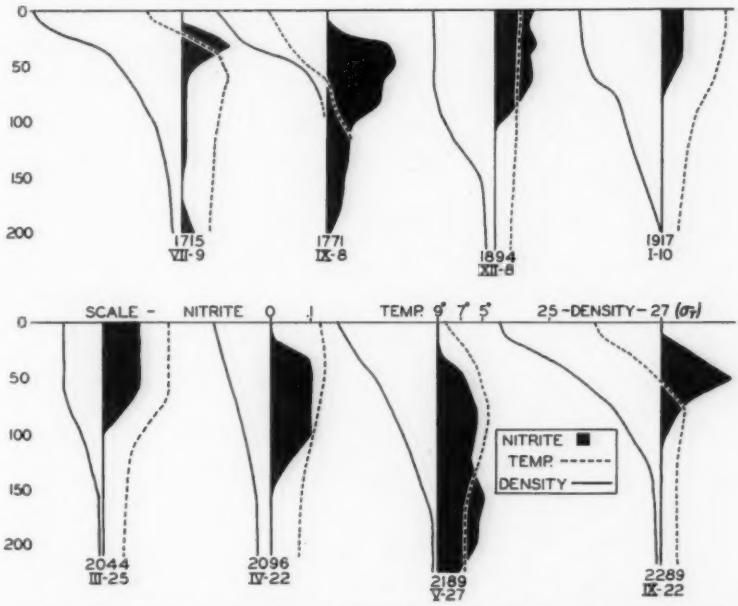

Fig. 12. Relation of nitrite to temperature and density (σ_t). Location 9, Gulf of Maine, at various times of year. Ordinates, depths in meters.

and nitrate. Figure 12 shows the progressive changes at Location 9 in a different way. The following points are revealed:

1. A minimum of nitrite occurs in January and February, concentrated at the surface. This corresponds to the period of maximum nitrate and phosphate, when the water mass is nearly homogeneous. Nitrification is going on at the surface, but only very slowly.

2. There follows a slow increase in nitrite until April, when more rapid production accompanies the diatom outburst and continues during the whole period of growth.

3. Simultaneously with the growth of diatoms, however, the removal of nitrate from the surface begins, and nitrite also starts to disappear in the same regions. At Locations 3 and 5 the removal of nitrite lags somewhat behind the nitrate, possibly because the latter is utilized more readily. At Location 7, during July, August and September of the second year, considerable nitrite is still present at the surface, but since the nitrate has not yet been completely used up it would seem that the nitrite store has not been heavily drawn upon.

4. The maximum of nitrite occurs between June and September, varying with local conditions at different stations. There is evidence that the seasons are somewhat displaced in different parts of the Gulf of Maine and that the growth of organisms does not take place uniformly throughout.

5. The utilization of nitrite in the surface layers, simultaneously with its production throughout a much deeper water mass, results in the appearance of a maximum zone, generally between 30 and 50 meters.

6. The occurrence of considerable nitrite in the surface layers at Locations 3, 8 and 15 late in October is noteworthy. This did not occur in all parts of the Gulf but does not seem to be fortuitous. It may well be associated with the death and disintegration of organisms after the close of the growth period. Soot-Ryen reported the same thing in his study of one of the Norwegian fjords. It is possible that this phenomenon was missed at Locations 5 and 7 from lack of data.

7. Location 22, being shallow, shows only the upper part of the picture. Maximum nitrite production has taken place here a little later.

The results of Soot-Ryen are altogether similar to these, with a slight displacement of the seasons. He found a minimum of nitrite in January, increasing through the spring and early summer to a maximum in August, but with depletion of the surface during the summer. This was followed by another short period of nitrite production in the upper layers in November.

Verjbinskaya's data on seasonal variation of nitrite in the Barents Sea are somewhat incomplete, but as far as they go they indicate the same general sequence: nitrite-free water in December and January, building up to a maximum in August, but with surface depletion during the summer.

Cooper's very complete study in the English Channel deals with a station too shallow to show vertical distribution of nitrite, but in all other respects is in complete accord with the above findings. It shows

the same simultaneous removal of nitrite and nitrate from the surface in the summer, followed by rapid production of nitrite and ammonia in October, and a minimum during December and January.

No study of seasonal variations at a truly oceanic station seems ever to have been made. Figure 8 shows the nitrite concentration in the upper 1,000 meters of the four corresponding stations in Group 3 (Table V). The only feature in common with the data from shallower stations is the surface depletion during the summer. Nitrite production evidently takes place in the upper levels during the winter. The hydrographic and biological conditions are so different here that a much more complete investigation is necessary.

The Position of Nitrite in the Nitrogen Cycle in the Sea

Brandt, in his monumental work on nitrogen metabolism in the sea, summarized all the earlier results and finally came to the conclusion that ammonium salts are oxidized to nitrite and nitrate by nitrifying bacteria in the sea bottom. Impressed by the fact that such organisms had never been found in the water itself, but that nitrite was frequently present at the surface, he proposed that bacteria might oxidize dissolved organic matter directly to nitrite and nitrate without ammonia as a necessary intermediate.

One view of the significance of nitrite was that expressed by Atkins: that its presence in sea water indicates the production of nitrate. Its absence from deep oceanic samples, on the other hand, shows that nitrification is complete. This assumes, of course, that nitrite is solely produced by oxidation of ammonia, or perhaps from dissolved organic matter directly.

Others, such as Brujewicz (1931) and Braarud and Klem (1931), have rejected this view and incline to that which explains nitrite formation as a result of the reduction of nitrate. Either theory will explain many of the results. Organic matter in the zone of nitrite production, present in considerable amounts, may either be the direct source of nitrite, with ammonia as a likely intermediate, or it may serve as the necessary source of energy for the nitrate-reducing bacteria. These have, in fact, been found there in small numbers.

The presence or absence of the necessary organisms is a bacteriological problem which this present paper cannot go into, but there are a number of considerations from the observed chemical data which favor oxidation as a source of nitrite, rather than reduction.

First, it is unlikely that the upper layers of the water, where oxygen tension is high, would be a suitable environment for reduction processes

to take place. The bacterial reduction of nitrate to nitrite is known to be possible under nearly anærobic conditions, and in the presence of enormous quantities of organic matter, but it is not certain that the same thing would take place in surface sea water.

The relation of nitrite and nitrate in bottom muds to each other and to the surrounding water, mentioned above, makes it clear that both are being *formed* in the bottom, obviously by oxidation of organic matter. Since nitrite is an intermediate in this process, it is scarcely possible that the large amounts found here could have been produced by a *subsequent* reduction of the nitrate; and if the nitrite in shallow muds is the result of oxidation it is very likely that all in the water above is of the same origin, for there is no evidence of any discontinuity of chemical mechanism from top to bottom. A comparison of the profiles of stations of varying depths suggests that the concentration of nitrite depends on a balance between the rate of chemical transformation and the rate of descent of the matter being transformed.

The relation between ammonia and nitrite which Cooper's results bring out, as well as the data presented above, especially the increase of both immediately after the summer growth period, are best explained on the assumption of oxidation of the former. The correspondence of the intermediate maximum zone of both nitrite and ammonia, already referred to, supports this view.

The general correlation of low concentrations of both ammonia and nitrite in the deep sea, and of higher concentrations of both, frequently, in other regions, is of some significance.

Furthermore, dissolved organic matter is nearly as abundant at great depths as near the surface, and nitrate is present in large quantity. This would seem to be an optimum condition for reduction of nitrate; but nitrite is seldom found here. It may, of course, be true that dissolved forms of organic matter are unsuitable for nitrate-reducing bacteria.

Finally, the following theory is presented to explain the observed facts:

During the winter, when biological activity is at a minimum, the decomposition of organic matter is going on very slowly. This takes place largely near the surface, either as the result of oxidation of excretory products liberated here, or the death of organisms, with disintegration and decay beginning before they have sunk far. Quantitatively, this same process goes on with greater rapidity as the season progresses. Nitrite is an early stage in the oxidation of this organic matter, presumably after the liberation of ammonia as a previous step. This is in keeping with Wattenberg's (1930) conclusion that ammonia formation is mainly a surface phenomenon. Later in the winter and

early spring, as the unstable column becomes deeper, these processes take place through a deeper vertical range.

Still later, when diatoms begin to grow actively they utilize the nitrite in the surface water, along with nitrate. Occasionally, when there are plenty of other nutrients, nitrite is not exhausted. Such is the case in the Antarctic, where determinations made on the "Discovery" show high concentrations of nitrite above 100 meters during the summer, but associated with very large quantities of nitrate and phosphate.

Organic matter works its way downward, by settling, if it is particulate, or by diffusion or mixing, if dissolved. The appearance of nitrite by oxidation therefore extends below the zone of its utilization. At greater depths it disappears again by further oxidation to nitrate.

Nitrate concentration continues to increase to still greater depths, reaching a maximum at 800–1,000 meters, generally. Nitrification extends further than the occurrence of nitrite would indicate.

The decomposition of organic matter takes place in several steps, and we may suppose that the products of the first stages are more rapidly oxidizable than those of the later ones. This may be the result of either the chemical nature of the material or its concentration, which would be greater at the start of the process, before it has become diluted. The formation of nitrite in significant amount is associated with the initial stages of the decomposition of organic matter, probably because of their greater rapidity, but the whole process of nitrification does not complete itself until greater depths are reached, and by this time the nitrite, formed in the first part of the process, has been further oxidized to nitrate.

Vertical differences in temperature may also play a part in determining the rates of decomposition.

Recently, ZoBell (1935) has suggested that diatoms may reduce nitrate to nitrite extracellularly and then absorb it as such. Such a theory would explain many of the observed phenomena with respect to nitrite distribution, to be sure, particularly its close association with phytoplankton. Its acceptance, however, will depend upon its satisfactory explanation of the relation of nitrite to ammonia. Such data as we now have covering this point seem difficult to reconcile with this view, but until we have had an opportunity to extend them we prefer to withold judgment.

ACKNOWLEDGMENT

I should like to express my thanks to Doctors H. P. Smith and H. E. Mahncke and Mr. E. F. Beach, who carried out some of the

analytical work, as well as to Doctors August Krogh and Ancel Keys, who furnished some of the ammonia results.

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THE BIOLOGY OF OITHONA SIMILIS IN THE GULF OF MAINE AND BAY OF FUNDY

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(*From the Woods Hole Oceanographic Institution* [1])

Introduction

Although *Oithona similis*, one of the two microcalanids found breeding in the Gulf of Maine, is small in size, the relative abundance of larvæ indicates that at times it forms a significant factor in the natural economy of the region.[2] It is also particularly favorable for quantitative study because adults, larvæ, and egg cases all appear to be sampled representatively with fine nets (No. 10 silk) and the pump.

This paper forms the third of a series on the biology of zoöplankton species in the Gulf of Maine and Bay of Fundy with special reference to production and dispersal, and is based on material obtained between July 28, 1931 and September 29, 1932, in the course of investigations carried on for the International Passamaquoddy Fisheries Commission. For a description of the area covered, location of stations, and methods, the reader is referred to a previous report (Fish, 1936*a*).

Regional Distribution of the Adult Stock

Oithona similis is almost "world wide" in distribution (Bigelow, 1926), but on the American coast appears to be largely centered north of Cape Cod. South from this point it is gradually replaced, in neritic waters at least, by *O. brevicornis*, a species not yet reported north of the Cape. At Woods Hole in 1922 and 1923 (Fish, 1925, Fig. 47) the latter species occurred with southern forms during the summer from late June to mid-October, and *O. similis* with the boreal population in autumn and winter. Farther south in Chesapeake Bay *O. similis* is decidedly a winter form limited largely to the inner Bay and is exceeded by *O. brevicornis*, which occurs everywhere in that region throughout the year (Wilson, 1932).

North of Cape Cod McMurrich (unpublished data) found *O. similis* in small numbers in Passamaquoddy Bay regularly during the colder months from November until March, but very rarely during the spring

[1] Contribution No. 109.

[2] *Microsetella norvegica*, the second species, is far less important numerically.

and summer. Elsewhere in Canadian waters, however, it has been reported widespread offshore during the summer (Herdman, Thompson, and Scott, 1898; T. Scott, 1905; Pinhey, 1926).

In 1931–32 *O. similis* was taken to some extent throughout the Gulf of Maine and Bay of Fundy, the most noticeable changes in the quantitative distribution of the adult stock occurring with the vernal warming of the water. In April, 1932 it was most numerous (up to 5,423 per minute) in the western area of the Gulf with smaller and apparently more local centers of abundance in the Middle Channel, off Yarmouth, and off Halifax. In the outer basin of the Gulf it was almost totally absent in the hauls (Fig. 1).

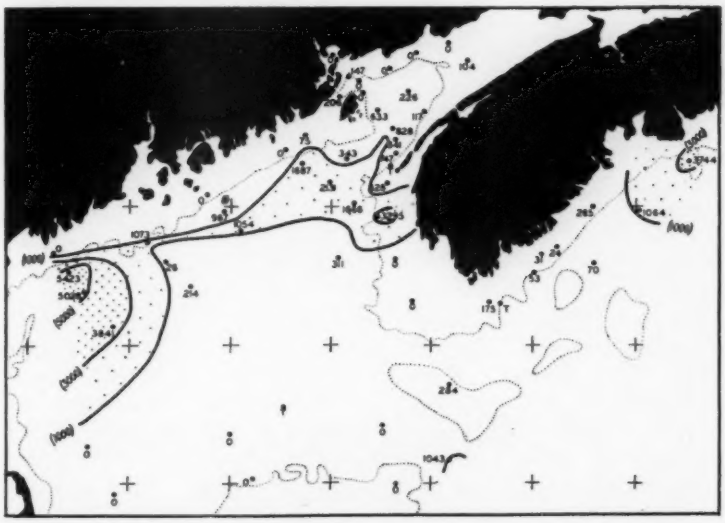

FIG. 1. Distribution of adults of *Oithona similis* in April, 1932. Number per minute of towing.

The changes in distribution from April to May would suggest that *Oithona* tends to withdraw into deeper water in winter, and approaches the coast with rising temperatures in the spring. By the end of May the greatest number of adults (6,087 per minute) was found at the innermost station off Casco Bay where the surface temperature was 8.63° C., although they were abundant throughout the western area (6.08°–9.38° C.) and in the Bay of Fundy along the course of the entering drift. The richest hauls at this time were everywhere ob-

tained at temperatures exceeding 6° C. *Oithona* was still sparse in the colder waters of the upper Bay, the Quoddy region, and thence westward along the coast of Maine to Frenchmans Bay.

There was a marked increase in the adult stock between May and June but no appreciable changes in distribution. Again the richest hauls were made nearest Casco Bay, and the poorest in the New Brunswick and eastern Maine coastal areas. The adult stock reached its peak at this time in the western Gulf and, although the subsequent crop yielded larger numbers of larvæ in August (Table IV), few apparently reached maturity. East of Mt. Desert adults did not reach their peak until August and the maximum was of short duration for, in the Bay of Fundy at least, they had almost reached the April level by mid-September.

Production and Dispersal of Eggs and Larvæ

Spawning Areas

Spawning is widespread but varies greatly in different parts of the region. Until July, in 1932, with the major portion of the *Oithona similis* population located in the western part of the Gulf, the rapid decline to the eastward resembled that of *Calanus finmarchicus* (Fish, 1936*a*, Fig. 11). Only in the outer part of the Bay of Fundy was there a slight break in the descending curve, and here the greatest increase was in copepodite stages, probably transported for some distance from the western or outer Gulf (Fig. 3). This is indicated by the low values in the sections from the inner Bay (*F* and *G*) and in the path of the drift from the Bay along the coast as far west as Mt. Desert (*E*).

The eastern region assumed greater importance in midsummer, and the curve for August (Fig. 3) more closely resembles that of *Pseudocalanus* (Fish, 1936*b*, Fig. 1) than *Calanus*. Extensive spawning continued in the western area, but the large numbers of early and late nauplii entering the Bay of Fundy and eastern Gulf at this time are indicative of important propagation nearby. Early nauplii, up to 19,258 per cubic meter, in the Bay could hardly have originated beyond the eastern basin. This is also evident from the number of early larvæ at all offshore stations east of Penobscot Bay in the Gulf (Fig. 9*C*).

The Bay of Fundy itself, however, appears to be a relatively unimportant spawning area. In the Quoddy region beyond the immediate influence of the drift from the Gulf, no eggs were found before July 30, and at all times both eggs and early stages were comparatively sparse, a condition also prevailing eastward along the New Brunswick coast and at the innermost stations (9, 10*A* and 11*A*). Elsewhere in the

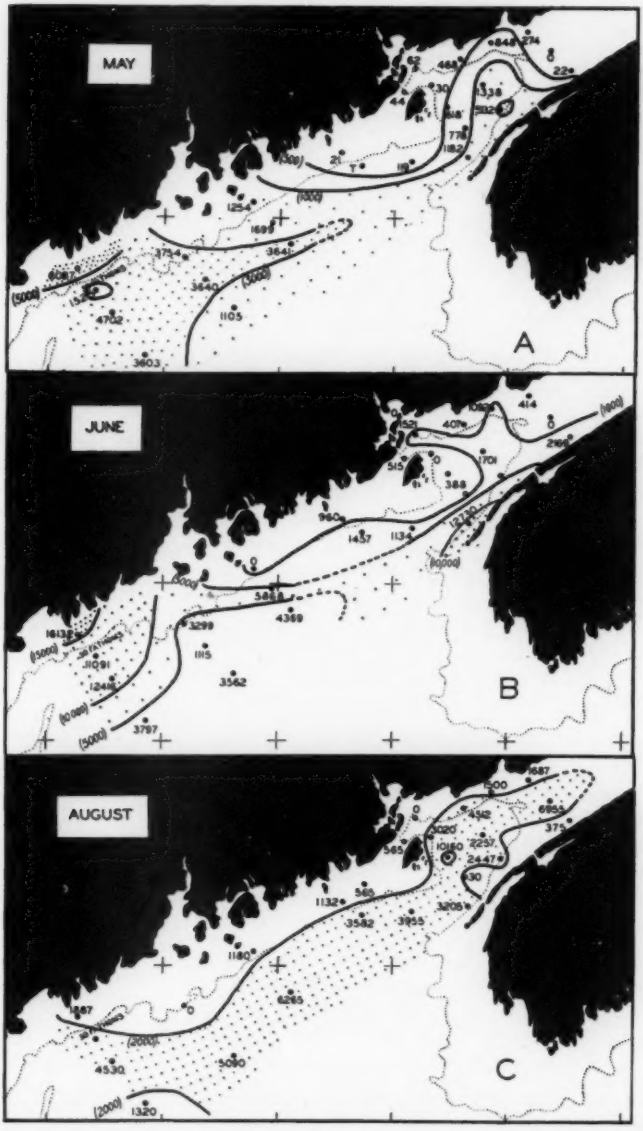

FIG. 2. Distribution of adults of *Oithona similis* in 1932. May–June: number per minute. August: number per cubic meter.

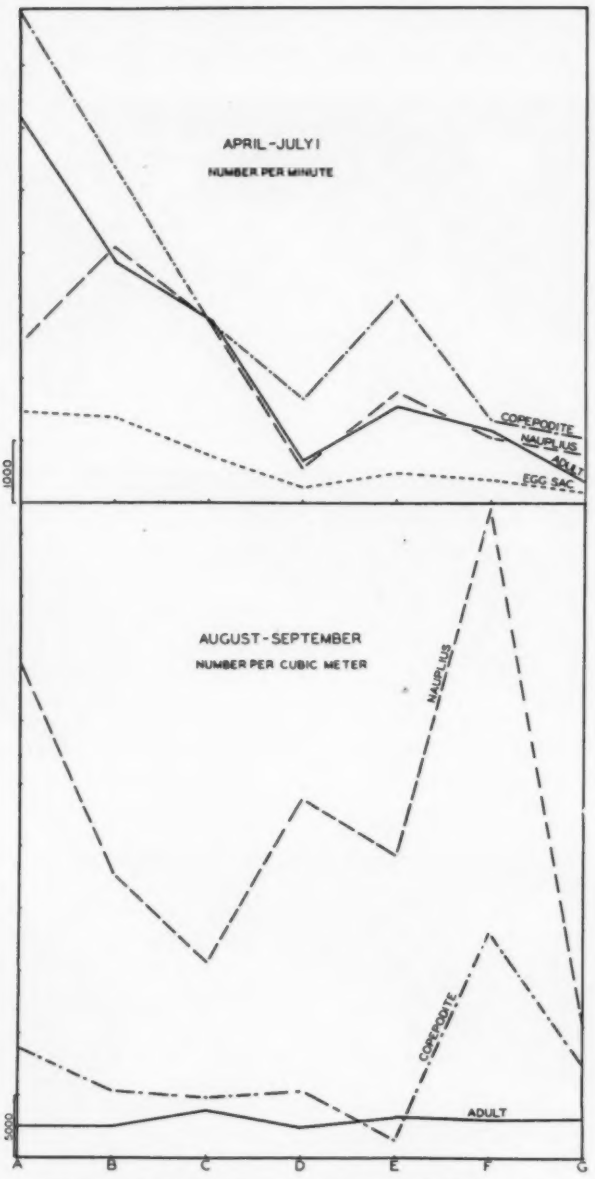

FIG. 3. Mean numbers of adults and young of *O. similis* in seven sections between Casco Bay and Cape Spencer in 1932. Sections: *A*. Casco Bay (Stations 23*A*–25*A*, 26); *B*. Penobscot Bay (Stations 27–29); *C*. Mt. Desert (Stations 17, 30–31); *D*. Moose Peak (Stations 32–33); *E*. Passamaquoddy Bay (Stations 5–8*A*, 35); *F*. Pt. Lipreau (Stations 13, 36–37); *G*. Cape Spencer (Stations 9–11).

Bay they were frequently very abundant, with hauls in August yielding up to 35,970 early nauplii per cubic meter (Fig. 9*C*), but these were always in the path of the entering drift.

Breeding Seasons

Like the other dominant boreal copepods, *Calanus finmarchicus* and *Pseudocalanus minutus*, *Oithona* begins spawning in the Gulf of Maine in March and continues in a succession of generations until September. There is a seasonal delay of about one month in the appearance of eggs in the eastern Gulf and Bay of Fundy and this regional variation permits the distinguishing of two breeding stocks, a western and an eastern. Also, as in the case of the two above-mentioned species it must be borne in mind that these stocks of *Oithona* are not permanently restricted to their respective localities, but merely represent delayed maturation of that portion of the winter population which happens to be located in the eastern part of the region during the spring, and earlier maturation in areas to the westward where the water mass responds more rapidly to vernal warming. However, since the augmentation starts at significantly different times in the two regions, with substantially the same interval of development, subsequent broods of the two stocks continue to appear at distinct periods irrespective of where they are dispersed.

Annual Cycle in the Two Breeding Stocks

Very little has been published on the biology of this species. Gran (1902) found some indication that the development of small species like *Oithona similis*, *Microsetella atlantica* and *Oncæa conifera* is completed in a much shorter time than in larger species like *Calanus finmarchicus*, *C. hyperboreas*, *Metridia longa*, and *Euchæta norvegica*.

Ruud (1929) reported that the stock of *O. helgolandica* (*similis*) doubled in number off More in 1925 from the end of May to the end of July. In 1926 a small stock at the beginning of March increased steadily until May 12. A second increase took place later, reaching a maximum on July 20. In 1927 conditions were similar except that the second maximum attained the highest peak of the year on July 31. Bogorov (1932) found copepodite stages III and IV predominating in the Barent's Sea at the end of July.

In the Gulf of Maine and Bay of Fundy the maxima of different larval stages, following the appearance of the first brood of the stock maturing in the western Gulf in March, indicate a developmental period throughout the region of about two months during the early season, and about six weeks in summer.

There appear to be at least three and possibly four broods of the western stock in March, May, July, and September. This stock, which in 1932 was represented by a maximum of late nauplius and copepodite

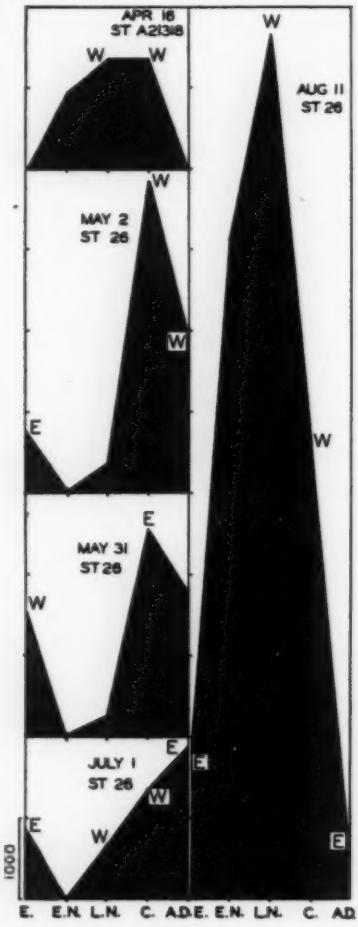

FIG. 4.

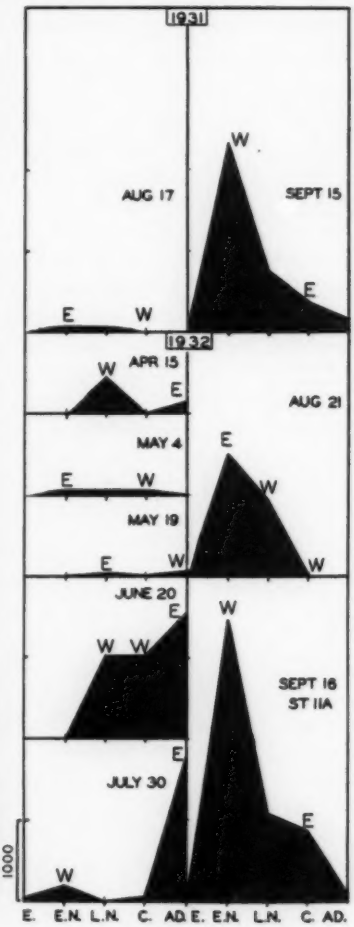

FIG. 5.

FIG. 4. Composition of the population of *Oithona similis* in the outer part of the western area in 1932, showing the succession of generations of the western (*W*) and eastern (*E*) stocks. April–June: number per minute. August–September: number per cubic meter. Stages recorded as *E*-egg sacs, *E.N.*—early nauplius (I–III), *L.N.*—late nauplius (IV–VI), *C.*—copepodites (I–V), *A.D.*—adults.

FIG. 5. Composition of the population of *Oithona similis* at Station 5 in the New Brunswick area of the Bay of Fundy in 1931 and 1932. Symbols as in Fig. 4.

stages on April 16, had by May 2 advanced largely to late copepodite stages and by May 31 matured and was spawning (Fig. 4). The second brood had reached late nauplius-copepodite stages by the end of June. The third brood, apparently developing more rapidly, was dominated by late nauplii in mid-August and what appeared to be a fourth brood was in egg and early nauplius stages on September 15–16 (Fig. 5).

There are three broods indicated in the eastern stock. Eggs found between April 20–May 2 had presumably advanced to copepodite stages by the end of May (Fig. 4). A second brood of eggs appeared a month later, and a third brood by mid-August. That portion of the stock remaining on September 15–16 in the Bay was apparently represented by late nauplius and early copepodite stages.

TABLE I

Dominant stages of the eastern and western stocks of P. minutus and O. similis occurring at comparable periods in 1932.

1932	Western Stock		Eastern Stock	
	P. minutus	O. similis	P. minutus	O. similis
April 16	Late nauplius	Late nauplius-copepodite	Egg	Egg
May 19–22	Late copepodite	Adult	Late nauplius	Late nauplius
May 31	Adult	Egg	Late nauplius-early copepodite	Copepodite
June 20–31	Late nauplius	Late nauplius-copepodite	Egg	Adult-egg
July 30	Egg—early nauplius	Early nauplius	Adult	Adult
August 11	Late nauplius	Late nauplius-copepodite	Adult-egg	Adult-egg
September 15–16	Egg	Egg—early nauplius	Late nauplius	Late nauplius-copepodite

The successive generations of the two breeding stocks of *Oithona* as shown in Table I and in Figs. 4 and 5 compare very closely with those of *Pseudocalanus* (Fish, 1936*b*, Figs. 2–4).

It will be noted that in the western stock *Oithona* in 1932 appeared to be slightly more advanced than *Pseudocalanus* at comparable periods throughout the summer, while in the eastern stock very little difference was detectable.

Productivity and Mortality in Developing Larvæ

Oithona similis was frequently taken during the breeding periods with one or two attached ovisacs. However, by far the larger number

was found floating free in the water. Whether or not the sac normally remains attached to the female for only a short period of time to insure fertilization, and then becomes detached to permit the formation of others, is not known. It is, of course, possible that those in the samples were broken off in the process of capture, but considering the large number of larvæ produced by a relatively small parent stock in August, several sacs of eggs must have been produced by each female at that season. Unlike *Pseudocalanus*, where the ovisac was never found around detached eggs, the ovisac of *Oithona* remains intact during the period of incubation and no doubt serves to protect the eggs from small predatory forms until they have hatched, a condition also found in *Microsetella*.

From April until the end of June in 1932 the stock of *Oithona* increased as shown in Table II, but considering the relative number of

TABLE II

Mean numbers of larvæ and adults of Oithona similis in the total region. April–June: number per minute. August–September: number per cubic meter.

	Month	Larvæ	Adults
1932	April	1,112	879
	May	5,918	2,519
	June	8,919	3,387
	August	34,852	2,938
	September	3,262	332
1931	August	33,925	3,550
	September	4,825	94

adults and young, it does not appear to have been an unusually prolific species during this period. In midsummer, however, with the maturing of the second western brood, a third crop of larvæ appeared, outnumbering the combined young of all other species of copepods both in 1931 and 1932 (Tables II and IV). It is not possible to say whether the parent stock of the third eastern brood is equally productive, because spawning had just started at the time of the last cruise in the Gulf, in mid-August.

Like *Pseudocalanus*, the greatest mortality in developing larvæ of *Oithona* seems to take place in the nauplius stages. This can be seen by comparing the abundance of nauplii in August with that of copepodites and adults in September (Fig. 9 and Table II). In the Bay of Fundy, where the mean number of late nauplius and copepodite stages in Au-

gust amounted to 26,655 per cubic meter, September collections showed a decline to 1,720 late larvæ and 332 adults per cubic meter. A similar decline was also found in September, 1931.

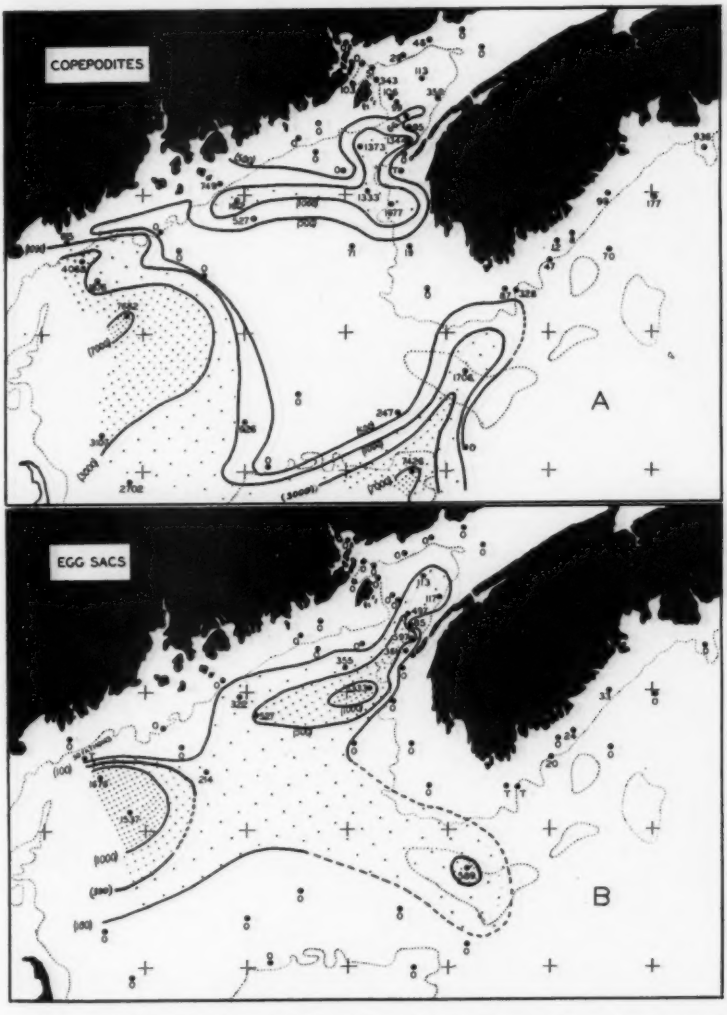

FIG. 6. Occurrence of copepodite stages of the western stock and egg sacs of the eastern stock in April, 1932. Number per minute of towing.

Dispersal and Regional Abundance of Eggs and Larvæ

Dispersal in 1932.—The method of tracing dispersal by monthly changes in the distribution of developmental stages of the different breeding stocks has been used in the present analyses. The general surface circulation and expected drift of eggs and larvæ within the Gulf and Bay of Fundy has previously been described (Fish, 1936*b*).

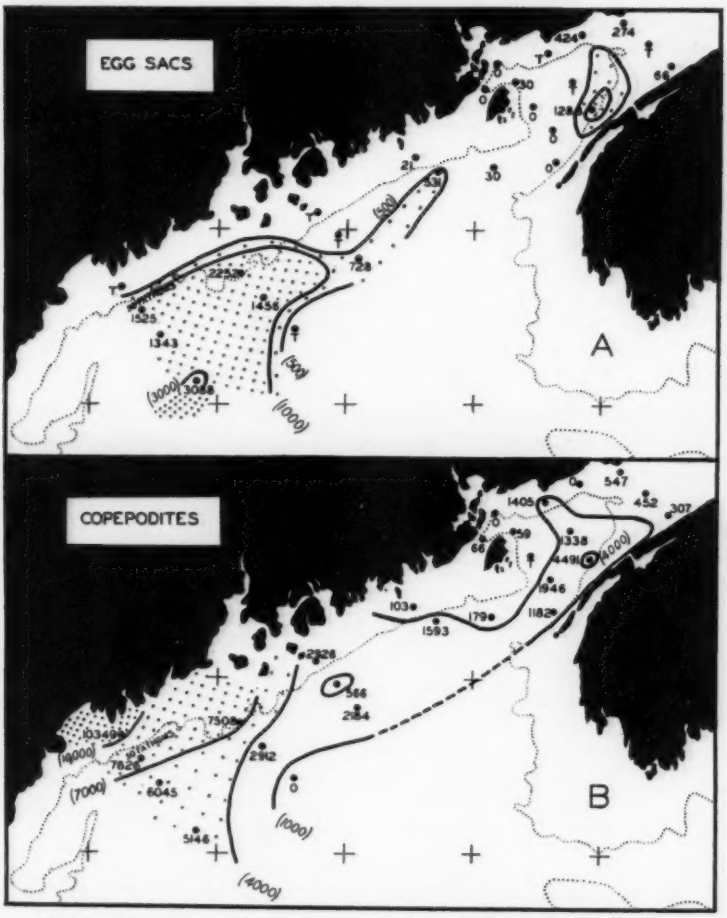

FIG. 7. Occurrence of egg sacs representing the second brood of the western stock and copepodite stages of the first brood of the eastern stock in May, 1932. Number per minute of towing.

In the case of *O. similis* there was an apparent difference in the general distribution of larvæ of the two breeding stocks. Spawning of the western stock began seaward of the 100-meter contour and subsequent generations remained for the most part concentrated offshore. The eastern stock, on the other hand, although emanating largely from the eastern basin, soon became widely dispersed in the coastal zone, and succeeding broods, particularly in the more advanced larval stages (Fig. 7*B*), were until August at times more abundant inside than outside of the 100-meter contour.

Western Stock.—Spawning offshore in the western basin and in the region of Georges Bank in March, by April 16 (Fig. 6*A*) larvæ in late nauplius and copepodite stages were found most abundant in the offing of Casco Bay and seaward in an arc corresponding with the general surface drift to the outer banks. Smaller numbers occurred in the eastern basin, the Bay of Fundy and along the south coast of Nova Scotia. Like *Calanus* and *Pseudocalanus*, in outer waters *Oithona similis* appears to reproduce off Halifax as early as in the western basin of the Gulf.

This brood remained centered in the western area, although by late May when the stock had matured and was spawning (Figs. 2*A* and 7*A*), a considerable number, having presumably circled the outer Gulf, were found entering the Bay of Fundy along the Nova Scotian side. The largest single haul of adults (possibly eastern stock; see p. 181) was made at the innermost station off Casco Bay, but egg sacs were for the most part concentrated offshore westward from Mt. Desert.

The second brood greatly increased the size of the western stock and by the end of June had become generally distributed offshore in the Gulf. The larvæ, at this time in late nauplius and copepodite stages (Fig. 8*A*), were abundant in all hauls seaward of the 100-meter line, particularly in the Casco section and along the Nova Scotian side of the Bay of Fundy. The fact that the numbers of both late nauplii (17,938 per minute) and copepodites (32,404 per minute) at the entrance to the Bay (Station 8*A*) exceeded those found anywhere in the inner Gulf may indicate that immigrants from the western area are supplemented in June by contributions from another important production area, probably in the vicinity of the outer banks.[3]

Once the second brood had become generally dispersed offshore very little change was noted thereafter in the relative regional abundance of comparable stages during the remainder of the summer. In mid-August copepodites of the third and largest brood (Fig. 9*B*) were distributed in

[3] Since immigrant larvæ of other species, abundant at the time, showed no evidence of unusual concentration at Station 8A (Fish, 1936*a* and *b*), this condition in the case of *Oithona* does not appear to have been due to local current action.

much the same manner as similar stages of the preceding brood in June (Fig. 8*A*), being abundant at all offshore stations. Late nauplii of the same brood (Fig. 9*A*) were somewhat more restricted, with concentrations west of Penobscot Bay and in the drift entering the Bay of Fundy.

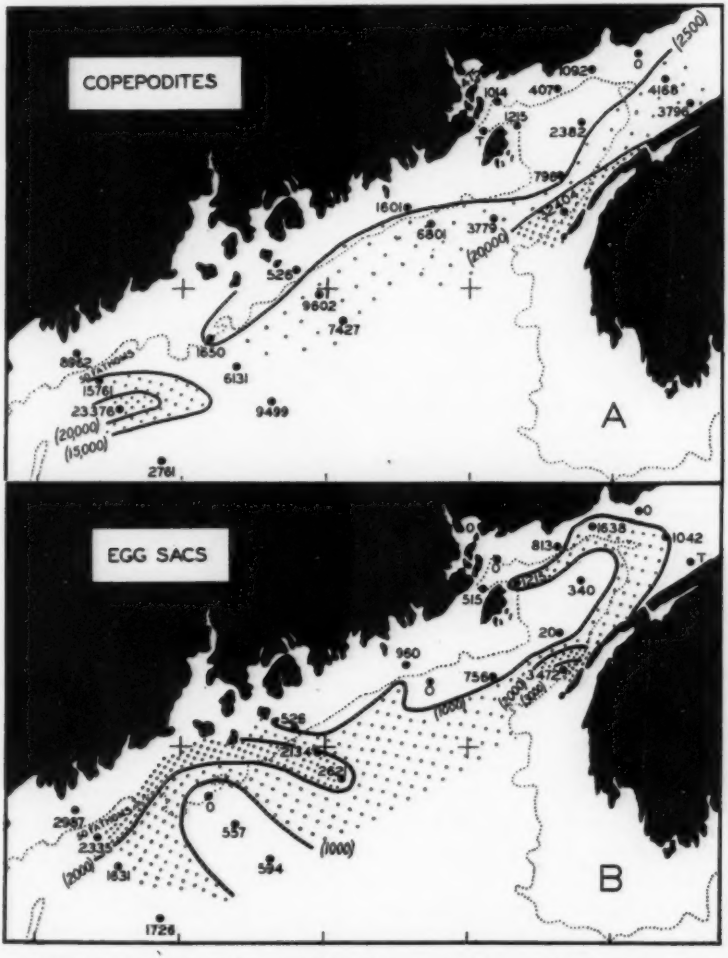

FIG. 8. Occurrence of copepodites of the second brood of the western stock and egg sacs of the second brood of the eastern stock in June, 1932. Number per minute of towing.

The coastal area east of Penobscot Bay and including the Bay of Fundy would appear to be relatively unproductive. The Bay apparently derives at times an abundant supply of nauplius stages from the eastern basin (Fig. 9*C*), but the eastern coastal region of the Gulf was found to be populated for the most part with a relatively small immigrant stock in more advanced stages (copepodites and adults). The large numbers of nauplii entering the Bay of Fundy along the Nova Scotian side must decline rapidly in the cold turbulent waters for there is little evidence that they survive the circuit of the Bay and are dispersed westward in the drift along the coast of Maine. The most probable source of the late stages found in the latter zone, east of Mt. Desert, is that portion of the drift from the outer Gulf and eastern basin passing westward across the entrance to the Bay.

By mid-September the *Oithona* population had greatly declined in the Bay of Fundy. Observations were not made in the Gulf after August, but in view of the absence of an appreciable influx in the entering drift it is probable that the Bay reflected conditions throughout the region.

Eastern Stock.—The first brood of the stock maturing in the eastern part of the inner Gulf and Bay of Fundy was just appearing near its southern and western margins in late April. Eggs were found in the eastern basin, over German Bank, and in a limited portion of the western basin, but where vernal warming was delayed in the more turbulent eastern coastal zone of the Gulf and the Bay there was as yet no evidence of propagation. Egg sacs west of Mt. Desert were all found in the drift from the eastward and were probably derived from a parent stock transported from the eastern basin (Fig. 6*B*). No eggs were found in the coastal zone anywhere in the Gulf.

By late May this first brood consisted largely of late copepodite stages which had become generally dispersed both in inshore and offshore waters, being most numerous well inshore in the western area (Fig. 7*B*). Some in the warmer western coastal zone had apparently passed through the final moult, for as indicated by the scarcity of eggs (Fig. 7*A*) at the innermost station off Casco Bay (Station 25*A*) adults there (Fig. 2*A*) were sexually immature and probably represented for the most part members of the eastern stock.[4]

Further evidence that only those eggs transported beyond the eastern coastal region before hatching survive, is seen in the composition of the eastern stock at this time. Since propagation had not begun in the inner Gulf east of Mt. Desert by the end of April, the product of this

[4] The western stock offshore had already spawned at this time (Fig. 7*A*).

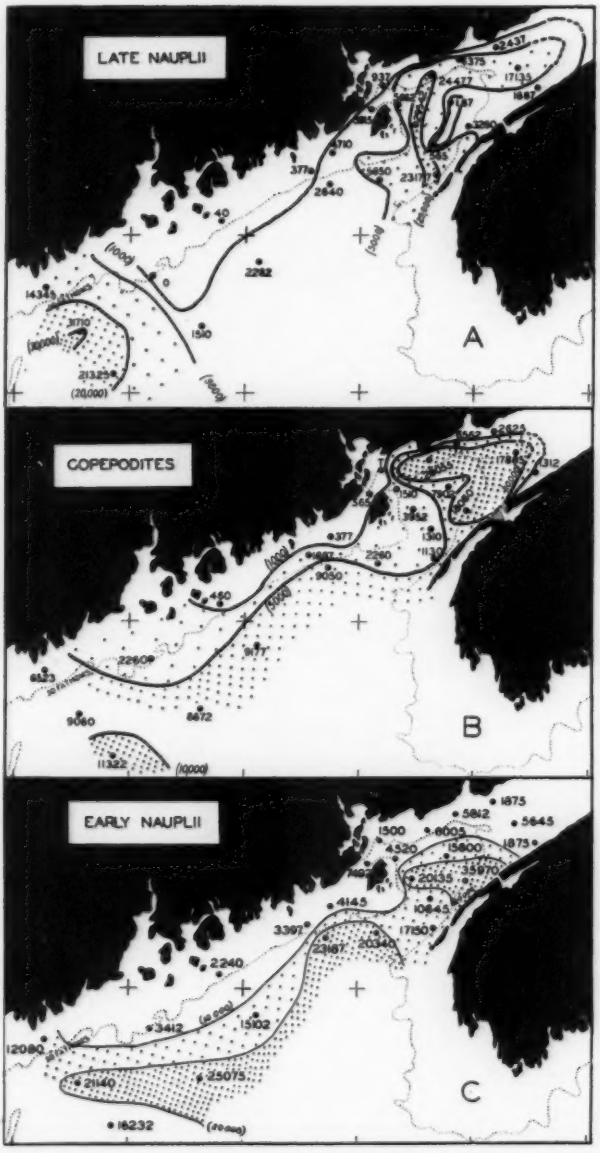

FIG. 9. Occurrence of late nauplii and copepodites of the third brood of the western stock, and early nauplii of the third brood of the eastern stock in August, 1932. Number per cubic meter.

late seasonal spawning a month later should have been represented by late nauplius stages, but very few were found. The young occurring in this region were copepodites assumed to be immigrants from more favorable production areas.

The distribution of eggs of the second eastern brood in late June (Fig. 8*B*) corresponded closely to that of the parent stock in the previous month (Fig. 7*A*). They were most numerous at the innermost stations in the Gulf and along the course of the drift into the Bay.

In mid-summer *Oithona* appeared to withdraw from the coast and the third eastern brood, in egg and nauplius stages, was found in August centered in the eastern basin and extending eastward into the Bay

TABLE III

Mean numbers of Oithona similis in the Gulf of Maine in 1931 and 1932. Number per cubic meter.

Stage	Gulf of Maine			
	Western Area		Central Area	
	1931 (Aug. 21–25)	1932 (Aug. 11–14)	1931 (Aug. 26)	1932 (Aug. 8–15)
Egg sac	1,947	1,855	1,409	1,224
Early nauplius	11,765	15,363	8,044	10,981
Late nauplius	12,018	15,529	21,133	11,175
Copepodite	7,908	7,217	9,220	5,144
Adult	3,537	2,557	3,575	3,090

of Fundy and westward along the outer stations to the western area (Fig. 9*C*).[5]

As in the case of the western stock, there must have been a great decline in the third eastern brood in late summer. In the Bay of Fundy by mid-September the survivors in late nauplius and copepodite stages were found entering along the Nova Scotian side in numbers not exceeding 3,000 per cubic meter.[6]

Annual Variation.—In the Gulf the mean numbers of larvæ and adults in August of the two years were of a somewhat similar order. In 1931 (August 21–26) there were 35,044 larvæ and 3,556 adults per cubic meter, and in 1932 (August 8–15), 32,705 larvæ and 2,824 adults. However, the mean for the Bay of Fundy at the beginning of September

[5] Within the region of observations in the Gulf in 1932, copepodites of the eastern stock in May (Fig. 7*B*) and eggs in June (Fig. 8*B*) were most abundant in the coastal zone westward from Mt. Desert.

[6] In August in this area early nauplii were found up to 35,970 per cubic meter.

in 1931 was more comparable with that of mid-September than of mid-August of the following year. In 1932 larvæ declined from 37,716 on August 17–21 to 3,262 per cubic meter on September 15–16, and adults from 3,120 to 332. The decrease had apparently already taken place in the Bay by September 1–5 in 1931, when the mean number of larvæ, 4,826, was almost as low as in the previous year in mid-September and the number of adults, 94, even lower.

Considered by areas, in the Gulf the total numbers and relative percentages in comparable stages exhibit no very striking differences (Table III). A similar condition in the case of *Calanus* (Fish, 1936*a*, p. 130) and *Pseudocalanus* (Fish, 1936*b*, p. 202) has been interpreted to mean that in 1931 the season was delayed about two weeks. Adults were slightly more numerous in both the central and western areas in 1931. The mean number of larvæ was higher in the western area in 1932, and in the central area in 1931.

TABLE IV

Mean numbers of larval stages of dominant species of copepods in the total region in 1932. April–June: number per minute. August–September: number per cubic meter.

1932	Calanus finmarchicus	Pseudocalanus minutus	Oithona similis
April	2,007	7,126	1,112
May	7,024	11,484	5,918
June	2,156	21,303	8,919
August	977	5,639	34,852
September	41	3,506	3,262
April–Sept.	(Mean) 2,441	9,812	10,813

RELATIVE NUMERICAL STRENGTH OF O. SIMILIS IN THE ZOÖPLANKTON POPULATION

Owing to its small size, *Oithona similis* is not taken in representative numbers in plankton nets of the type usually used in American waters and for this reason former available records do not indicate accurately its relative abundance in the boreal population of the western Atlantic. Its presence in large numbers in collections from the Newfoundland region whenever there was a considerable amount of ctenophore debris (Pinhey, 1926) would indicate that it is abundant in those waters in late summer, but was captured only when the meshes of the net became partially clogged. As far south as Chesapeake Bay *O. similis* has been found by Wilson (1932) to form an important addition to the winter plankton. In the eastern Atlantic Gran (1902) reported that this spe-

cies also plays an important rôle off Norway, and Ruud (1929) in the same region found it comprising up to 60 per cent of the copepod stock in July, 1925.

In the present investigations *Oithona similis* rarely appeared in the coarse meter net samples, but an estimate of its importance in the region during the spring and summer months can be obtained by comparing the relative abundance of larvæ with those of other dominant species. In making such a comparison it must be borne in mind that the relative numbers of young do not necessarily indicate that adults occur in the same proportion. The mortality of developing young appears to be very much greater in some species than in others and, like *Pseudocalanus* (Fish, 1936*b*), a very small percentage of *Oithona* larvæ apparently reach maturity. Again, because of its small size *Oithona* in the adult form must rank far below *Calanus* and *Pseudocalanus* in importance. However, it is not the adult stock of *Oithona* but the tremendous numbers of larvæ produced by this prolific species which appears to be of greatest significance in the economy of the region. Particularly in August it must form a most important source of food for those species feeding on larval copepods. In Table IV it will be seen that, based on the mean numbers of young between April and September (1932), *Oithona* ranks with *Pseudocalanus* and far exceeds *Calanus*.

Of added importance in the economy of the region is the fact that the seasonal maxima of the three species occur at successively later periods during the summer. Whereas *Calanus* reaches its peak in May (first brood), *Pseudocalanus* is most abundant in June (second brood), and *Oithona* (third brood) in August.[7] In the latter month the mean of *Oithona* larvæ for the entire region amounted to 34,852 per cubic meter.

Summary

1. *Oithona similis*, although almost world-wide in distribution, is centered largely north of Cape Cod on the western Atlantic coast, and south from this point is gradually replaced by *O. brevicornis*.

2. The adult stock in the Gulf of Maine appears to approach the coast from offshore with rising temperatures in the spring.

3. Propagation begins in the Gulf in March and continues in a succession of generations until September.

4. Two breeding stocks are distinguishable, due to an average difference of about one month in the time of vernal propagation in the

[7] To this seasonal progression may be added a September maximum of *Centropages typicus* larvæ throughout the Gulf and Bay of Fundy.

eastern and western parts of the region. This difference is reflected in subsequent generations after dispersal.

5. There appear to be four broods of the western stock, in March, May, July, and September, and three in the eastern, in April, June, and August.

6. A developmental period of two months during the early season and about six weeks in summer is indicated.

7. *Oithona* is at times far more prolific than either *Calanus* or *Pseudocalanus,* but mortality is relatively much higher, depletion being greatest in nauplius stages.

8. The western stock remained for the most part concentrated offshore, but the eastern, particularly in advanced stages, was more generally distributed, sometimes being more abundant inside than outside of the 100-meter contour.

9. There is some evidence that in June the local stock in the inner Gulf is supplemented by important contributions from another production area, probably in the vicinity of the outer banks.

10. There is no indication of successful propagation in the turbulent coastal region of the Gulf east of Mt. Desert or in the Bay of Fundy.

11. The larvæ of *Oithona* afford the most abundant source of food in the region in mid-summer for those animals feeding on larval planktonic organisms. Of added importance in the natural economy of the region is the fact that the seasonal maxima of the three numerically dominant species occur at successively later periods during the summer, *Calanus* in May, *Pseudocalanus* in June, and *Oithona* in August.

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THE EFFECT OF ULTRACENTRIFUGING UPON CHICK EMBRYONIC CELLS, WITH SPECIAL REFERENCE TO THE "RESTING" NUCLEUS AND THE MITOTIC SPINDLE[1]

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Few, if any, problems of the cell are more perplexing to the cytologist than those of the structure of the "resting" nucleus and of the mitotic figure. Some of the structures involved can be seen in the living cell, but for others we must rely almost exclusively upon fixed and stained preparations for our information. Because of this fact, marked difference of opinion exists concerning those structures that can only be seen in the fixed preparations; many investigators consider them artifacts which have no counterpart in the living cell.

Within the past four years, work in this laboratory with the ultracentrifuge has to some extent concerned itself with the problems of the physical nature of protoplasm, particularly its components and inclusions in certain somatic tissues (Beams and King, 1934*a*, *b*; 1935). In the present study we propose to limit our description mainly to the effects of ultracentrifuging upon the "resting" nucleus, chromosomes, and mitotic apparatus, hoping thereby to contribute to the knowledge of their physical constitution.

Material and Methods

The method of centrifuging is, for the most part, similar to that already described (Beams and King, 1934*a*). Forty-eight to 96-hour-old chick embryos were removed from the egg and placed in physiological salt solution warmed to 102° F. They were then immediately transferred in the physiological solution to the rotor of the air-driven ultracentrifuge, where they were whirled for 10 minutes at approximately 150,000 times the force of gravity and immediately thereafter fixed in Bouin's solution. Sections were cut and were stained in Delafield's or in Heidenhain's hematoxylin.

Description

In Fig. 1 is shown a mesenchyme cell of the sclerotome region of an 80-hour chick embryo, illustrating the usual position of the inter-

[1] Aided by grant from the Rockefeller Foundation for work on cellular biology.

kinetic or "resting" nucleus and the normal distribution of its various components, namely, nucleoli, chromatin, "reticulum," nuclear "sap" (karyolymph) and nuclear membrane. It is quite evident that in the nuclei of these, unlike the condition for other cells (Herrick, 1895; Gray, 1927), the distribution of the various components is not noticeably affected by the normal pull of gravity. However, when the force of gravity is increased 150,000 times, a marked displacement and stratification of the nuclear materials in the order of their relative specific gravity is evident, as illustrated in Figs. 2, 3, 4, and 5. In Fig. 2 the nucleolus is shown displaced to the heavy or centrifugal pole, the chromatin (basichromatin) and "reticulum" or "linin" (oxychromatin) materials are next in order and the non-staining nuclear "sap" or karyolymph is concentrated at the lighter or centripetal pole of the nucleus. In this preparation the nucleus as a whole has not been greatly displaced within the cell. However, in Figs. 3, 4, and 5, a progressively more extensive effect is evident in that the nucleus is stretched in a direction parallel with that of the centrifugal force. The condition in Fig. 5 is extreme, as the nucleus has been extended to over four times its normal length; this illustrates beautifully the elastic properties of the nuclear membrane and the materials included in it. We have never encountered in any of our experiments evidence to support the view that the nucleus is anchored in position by cytoplasmic strands from the cell membrane, as has been figured for cells from the hair of the squash by Heidenhain (1907).

A somewhat different effect is seen in the resting nucleus of the red-blood corpuscle, where a separation of the chromatic from the achromatic elements of the nucleus is effected (Fig. 10). The darkly staining mass at the centrifugal pole contains the chromatic moiety while the rounded clear body at the opposite or centripetal pole is the achromatic or nuclear "sap" portion.

This condition has been noted by us in centrifuged paramecium (King and Beams, 1934): such animals have been observed to live for several days, but apparently do not divide normally (unpublished data). This phenomenon clearly demonstrates that, in these cells too, the chromatic elements of the nucleus are distinctly heavier and the achromatic materials lighter than the cytoplasm.

In reference to the spindle in the following description of dividing cells it is evident that we are dealing with what Schrader (1934) has termed half-spindle and continuous fibers. However, since our material is not favorable for a careful differentiation of these components, we are not here concerned with the origin of the spindle components but will simply speak of them collectively as spindle fibers.

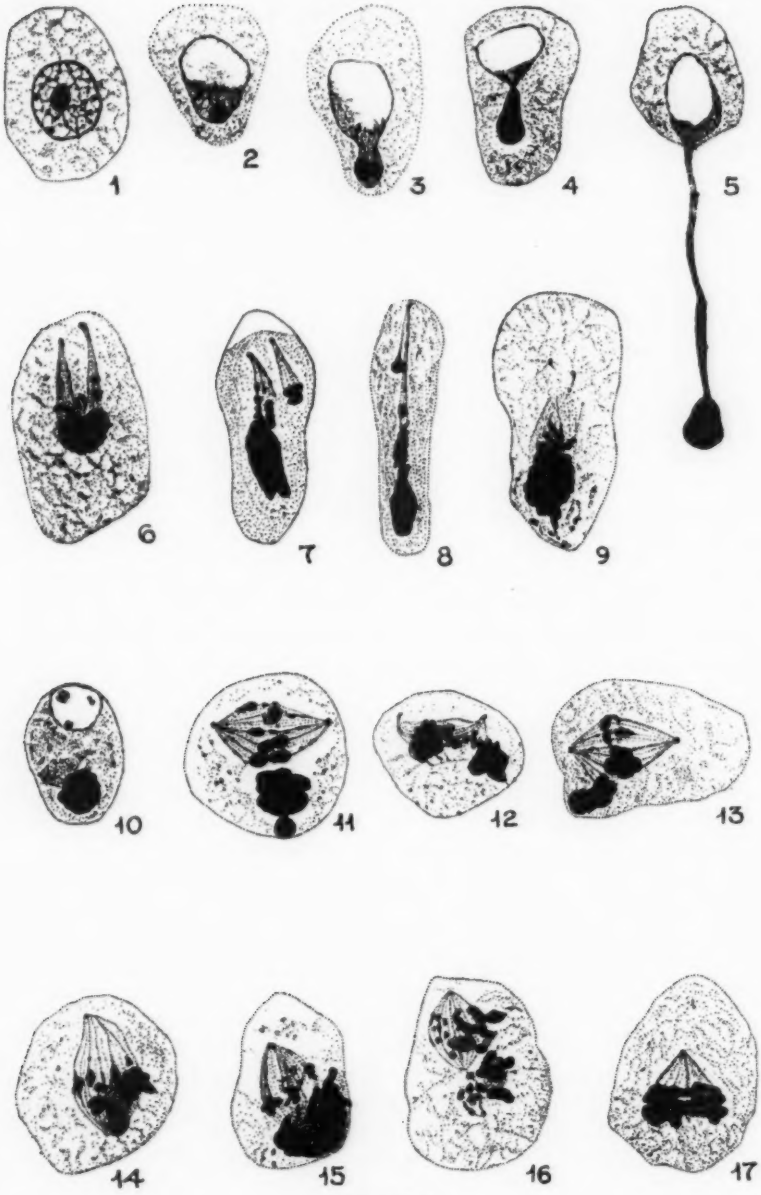

PLATE I

Many different effects of centrifugal force upon the metaphase mitotic spindle are noted, depending to a large degree upon the orientation of the spindle as regards the direction of the force. For instance, in Figs. 6, 7, 8, and 9, the force has been applied at right angles to the spindle and rather marked effects are observed. In Fig. 6, instead of the mitotic spindle being displaced *in toto* through the cytoplasm of the cell as is often the case in other material (Lillie, 1909; Spooner, 1911; Morgan, 1927), the two halves of the spindle with their associated centrioles are often stretched and bent centripetally through an angle of approximately ninety degrees; the attached metaphase chromosomes in this particular case have not been greatly displaced, nor has the spindle returned to its normal position after release of the centrifugal force. However, in Figs. 7, 8, and 9, similar but somewhat more marked effects upon the spindle are noted. In Figs. 7 and 8 the bulk of the chromosomes has been displaced centrifugally and torn free from one half of the spindle; the other half of the spindle is greatly stretched and apparently was in the process of separating from the chromosomes when the centrifuge was stopped. It is of considerable interest that after such treatment the detached halves of the spindle still possess well-

EXPLANATION OF FIGURES

All the drawings were made by Miss Gladys Larsen from mesenchyme cells of approximately 80-hour-old chick embryos unless otherwise indicated. The figures have been magnified approximately 2,000 times. The direction of the centrifugal force in all cases has been toward the bottom of the plate.

FIG. 1. Control cell showing the usual distribution of the "resting" nuclear elements.

FIGS. 2, 3, 4, and 5. Centrifuged cells showing a progressively more drastic effect upon the "resting" nucleus and illustrating the relative specific gravity and elastic properties of its materials.

FIGS. 6, 7, 8, and 9. Cases illustrating the bending and stretching of the spindle toward the centripetal pole, displacement of the chromosomes from the metaphase plate centrifugally and disintegration of the spindle, leaving the centrioles free in the cytoplasm.

FIG. 10. Blood corpuscle. The nucleus has been separated into a chromatic portion (at the centrifugal pole) and an achromatic portion (at the centripetal pole).

FIGS. 11, 12, and 13. Cases depicting the displacement of portions of the chromosomes from the spindle. Always a portion of each chromosome remains attached to the spindle fibers. Separation of some of the spindle fibers are also observed.

FIG. 14. Cell showing displacement of the chromosomes along the centrifugal half of the spindle.

FIG. 15. Cell showing displacement and apparent disintegration of the centrifugal half of the spindle.

FIG. 16. Cell with its spindle displaced to the centripetal pole. Portions of the chromosomes have been torn from the spindle.

FIG. 17. Cell with an apparently normal centripetal half of a spindle, but with the lower centrifugal half missing.

defined centrioles and spindle fibers. Here, as in other cases to be mentioned later, fragments of the chromosomes still remain attached to the spindle fibers; this definitely indicates that the union between spindle substance (fibers) and chromosomes is stronger than the internal cohesion of the chromosomes. Furthermore, it is of interest that, when they are free of most of their chromosome material, the half spindles move readily to the centripetal pole of the cells, and hence must be lighter than the surrounding cytoplasm. In the final stages the entire achromatic spindle, save for the centrioles, frequently undergoes a marked physical disintegration (Fig. 9). Only faintly can one observe a portion of the spindle fibers on the centripetal side of the chromosome mass. The two centrioles seem to be completely detached from the spindle substance and are free to move in a centripetal direction. They are not, however, as near the centripetal pole of the cell as might have been expected from the condition in Figs. 7 and 8.

In still other material subjected to the same treatment, cells may be found where most of the chromosomes have been thrown off the equatorial plate of the spindle into the centrifugal end of the cell; the spindle remaining apparently normal in the centripetal end of the cell with very small portions of the chromosomes still attached at its equatorial plate (Fig. 11). Probably the spindle has been greatly distorted, as shown in Fig. 6, during the action of the force, and subsequently has returned to normal upon cessation of centrifuging. Frequently, in similarly treated cells, the spindle has been separated, since part of the spindle fibers can be seen to be still attached to the displaced chromosomes (Figs. 12 and 13). The presence of fragments of chromosomes remaining attached to the spindle at the equatorial plate after such treatment strongly suggests that the spindle and the union between the spindle substance and chromosome is able to withstand greater distortion before breaking than the substance of the chromosomes themselves.

When the mitotic spindle is oriented approximately parallel to the centrifugal force, quite different effects are found. In Fig. 14 the force has acted slightly obliquely from the upper left, forcing the bulk of the chromosomes centrifugally and to the right side of the lower half of the spindle. Here too, as in other cases mentioned above, the fiber attachment portions of the chromosomes are not displaced from the equatorial plate. Sometimes most of the chromosomes are thrown completely free from the spindle into the centrifugal half of the cell (Fig. 15). We were unable to determine whether or not the centrifugal portion of the spindle in this instance has disintegrated or is only masked by the displaced chromosomes. In any case, the centripetal half of the spindle seems to be normal and to possess the usual chromosome fragments on

the equatorial plate at the points of spindle attachments. Other cells, treated in a like fashion, often have only part of their chromosomes displaced from the spindle (Fig. 16). In such cases the intact spindle, with its remaining chromosomes and fragments of displaced chromosomes, usually moves into the centripetal half of the cell, the displaced chromosomes to the centrifugal half. Finally cells are found which have a normal centripetal half of a spindle with its metaphase plate of chromosomes while the lower or centrifugal half of the spindle is apparently absent altogether (Fig. 17). This condition is strikingly similar to that recently reported for the onion root tip by Luyet (1935). In such a case, the question immediately arises whether the lower half of the spindle has been forced centripetally, as is known to be the case in Fig. 6, and subsequently lost to view, as has been suggested by Luyet; or whether it is no longer distinct from the surrounding cytoplasm, due perhaps to the pressure developed between the chromosomes and the cell membrane. We are inclined to accept the latter view, because we have never seen the lower half of the spindle in the large number of such cases examined. In this connection the observations of Harvey (1935) are of interest: she observed that the mitotic spindle of certain sea-urchin eggs moved to the centripetal pole upon centrifuging and that the astral rays became much shortened on the compressed or centripetal side of the aster.

The question as to the survival of these chick cells has not been satisfactorily answered as yet. We hope that sometime in the near future attempts may be made to grow centrifuged chick cells in tissue cultures. However, we already know that root tip cells of wheat seedlings do recover after similar distortion by ultracentrifuging.[2]

Discussion

Notwithstanding the fact that thousands of papers have appeared describing and illustrating the nucleus in practically every type of cell since it was first discovered by Robert Brown in 1831 (Wilson, 1925), there still remains no general agreement as regards its real physical constitution. For example, one reads in Wilson's " The Cell in Development and Heredity," that the resting " vesicular nucleus, as seen in sections, usually shows four distinct components, namely: an enclosing wall or membrane; a nuclear framework usually described as a network or reticulum, though by some observers regarded as an alveolar struc-

[2] Eggs of *Ascaris suum* will recover and divide after ultracentrifuging at 400,000 times gravity for an hour; we have recently centrifuged *Ascaris* eggs at 150,000 times gravity for 10 days during which time they divided. (In press, Science, 1936.)

ture; the nuclear sap, enchylema, or ground substance which occupies the interstices of the framework; and one or more nucleoli, massive and usually rounded bodies suspended in the framework." On the other hand, some investigators express the opinion summarized by Gray (1931) in his "Textbook of Experimental Cytology" that "Since all nuclei exhibit a visible granular or fibrillar structure after coagulative fixation, it is generally supposed that the structures seen in preserved preparations or in moribund nuclei are to be regarded as purely artificial products of coagulation, which cannot be correlated with the fundamental structure of the living nucleus. This view, developed many years ago by Hardy (1899), is now accepted by the majority of animal cytologists (Champy, 1913; Policard, 1922; Lewis and Lewis, 1924; Chambers and Rényi, 1925; Schitz, 1925; Burrows, 1927)."

It will be clear to those who analyze the literature on the physical nature of the nucleus that those who support the former view are mainly morphological cytologists, who have worked chiefly with fixed materials (however, see Bělař, 1929; Cleveland, 1935) and that those who support the latter view are chiefly experimental cytologists, who have worked mainly with surviving cells. In other words, the question resolves itself into whether or not the chromatin, "reticulum," nucleolus and nuclear "sap" seen in fixed preparations of the "resting" nucleus, correspond to the condition in the living nucleus to the same degree as do the chromosomes and other elements of the dividing nucleus.

The results reported in this paper seem to bear upon these questions, insofar as we have demonstrated that after centrifuging the living nucleus and immediately fixing and staining it, all the structures mentioned above are present, but displaced and stratified in the order of their relative specific gravity. This can only be interpreted to mean that the nucleus is not a homogeneous body, although it often appears so in the living material. It means, we think, that it is composed of materials of different relative density, yet of similar index of refraction, which usually prevents its elements from being seen in the living nucleus. Moreover, it is significant that these displaced nuclear elements appear morphologically similar in structure to those of the uncentrifuged fixed nucleus. Furthermore, the chromatin and "reticulum" materials in the centrifuged and subsequently fixed preparations take up the same relative position in the nucleus as does the prophase chromatin and chromosomes and hence must be of a similar specific gravity. Therefore, these results as well as the observations of the majority of cytologists have convinced us that well-fixed preparations of the "resting" nucleus probably give as reliable a picture of the condition during life as do fixed preparations of the prophase nuclei and chromosomes.

We, of course, realize that the structure of the "resting" nucleus no doubt varies greatly in different types of cells. For example, the chromatin in the nuclei of certain insect ova (Gardiner, 1935) is very scarce (diffuse?) as determined by the Feulgen method. In contrast to this, however, the nuclei of the salivary glands of many dipterous larvæ give a very marked reaction as of very orderly arranged segments of chromatin on an achromatic framework ("reticulum"). It would seem almost incredible that in the nuclei of the salivary glands of, for instance, *Chironomus* larvæ (King and Beams, 1935), all the material of the nuclei, except for the nucleolus, is evenly dispersed in a homogenous mass throughout the nucleus and subsequently clumped by fixation into long chromosomes, the identity of which may be recognized from cell to cell. In this connection, Doyle and Metz (1935) have recently shown, in the living nucleus of the salivary glands of *Sciara* larvæ, that no chromosomes or chromatic segments, so characteristically seen in aceto-carmine preparations, are to be observed. They nevertheless conclude from their studies that the chromosomes and chromatin segments are always present in the living nucleus, but that their refractive index is such that they are not optically differentiated.

Němec (1929) concludes from his work on centrifuged plant nuclei that a definite "reticulum" is present which is attached to the nuclear membrane. From our studies we cannot be sure just what constitutes the so-called "reticulum"; that is, whether it represents an achromatic framework in the nucleus impregnated with chromatin or whether it consists entirely of chromatin material. We are inclined to think that the "reticulum" in preserved cells has not been precipitated by fixation from a homogenous mass, but that it corresponds to a special material similarly distributed in the living "resting" nucleus.

When an inquiry is directed toward the structure and function of the mitotic figure, particularly the centrioles and spindle, one encounters an enormous amount of literature containing a most extraordinary group of conflicting contentions (see Wilson, 1925; Sharp, 1934; Schrader, 1934 and Bleier, 1930, for a review of the literature). It is beyond the scope of this paper to deal generally with a discussion of the mechanism of mitosis, but our results do seem to bear upon certain points of the structure of the mitotic apparatus which will be discussed below.

The reality of centrioles in living cells has recently been seriously questioned by Fry (1929). However, the centrioles in our material still possess their typical characteristics after being moved through the cytoplasm to the centripetal pole of the cell where they are in some cases detached and rest freely in the cytoplasm. This we believe is

good evidence for their existence in the living cell. Furthermore, the fact that they have been so clearly demonstrated in the living cell by Cleveland (1935) and their genetic continuity so carefully followed by Heuttner (1933) leaves little doubt that they are actually present.

As regards the spindle, one group of investigators of which Chambers (1924) and Lewis and Lewis (1924) are typical, holds that the mitotic spindle is optically homogeneous, and the appearance of fibers in fixed preparations is an artifact, without any correspondence to structures in the living cell. However, in contrast to this view is the one derived by Cleveland (1935) from his work on living protozoan cells. Here "there is not the slightest doubt regarding the existence of the centrioles, the formation of the achromatic figure from the centrioles, the fibrillar nature of the achromatic figure, and the rôle of the achromatic figure in nuclear division." Our work points to the fact that the spindle and centrioles in chick cells are, in general, distinctly lighter than the chromosomes and for the most part lighter than the surrounding cytoplasm, as is evident by their frequent movement to the centripetal pole of the cell. The fact that the spindle is capable of considerable bending and distortion, without becoming detached or dissolved from the chromosomes or centrioles, argues that it is a body of considerable form and structure rather than an artifact. However, from our work we cannot definitely say that fibers are actually present in the living spindle, but certainly the structure of the spindle is capable of considerable tension and plasticity; it may be partially split and still possess its typical appearance in fixed preparations. These observations, we believe, support those of Cleveland (1935), who was able to establish by pulling the centrioles that the spindle fibers are real and definitely attached to the chromosomes. Recently Schrader (1934) has centrifuged dividing cells of crustaceans, molluscans and insects, with much less force than used by us. He states that "metaphases so treated continue their mitotic activities and it is concluded that the half-spindle components as seen in fixed preparations are not coagulation artifacts but have a morphological basis in the living spindle." Furthermore Wyckoff (1934), who attempted to photograph (with ultraviolet light) the living spindle, says "you surely see something; but whether it is the individual fibers or the result of a state of strain in the viscous protoplasm, I don't know." Lewis (1934) has recently reported that when the metaphase spindle of chick cells in tissue culture is exposed to a hypotonic medium, the fibers disappear, the chromosomes are dispersed from the equatorial plate and the progress of division stopped. However, if the hypotonic medium be replaced in time by an isotonic

medium, a new spindle forms, the chromosomes collect on the equatorial plate, and division follows. If the cell is fixed and stained after the action of the hypotonic medium but before the addition of the isotonic medium, no spindle fibers are to be found, although the centrospheres seem not to have disappeared. These observations indicate definitely that the spindle is essential for division in chick cells, and that it is capable of being dissolved and reformed while the cell is in the metaphase state of division. In any case, whatever the structure of the spindle may be, we are in agreement with Seifriz (1929) " that it makes little difference whether we regard the spindle fibers as threads, rows of granules or lines of force. If fixation causes previously existing lines of force to appear as fibers this is sufficient, for, after all what are lines of force if not the linear orientation of particles, whether electrons or protoplasmic granules."

Conclusions

1. Chick embryonic cells, both " resting " and in stages of division, have been centrifuged at approximately 150,000 times gravity for 10 minutes.

2. The elements of the " resting " nucleus were stratified in the order of their decreasing specific gravity as follows: (*a*) nucleoli; (*b*) chromatin and " reticulum "; (*c*) nuclear " sap."

3. Evidence is presented to support the view that fixed preparations of the " resting " nucleus give as characteristic a representation of the elements during life as do fixed preparations of prophase nuclei and metaphase chromosomes.

4. The metaphase spindle has been distorted, its chromosomes displaced, its fibers separated, and, in some cases, it has been completely disintegrated by centrifuging.

5. These results support the claim that spindle fibers and centrioles as seen in fixed preparations have a morphological basis in the living spindle.

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THE EFFECTS OF HEAVY WATER UPON THE FISSION RATE AND THE LIFE CYCLE OF THE CILIATE, UROLEPTUS MOBILIS

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Since the discovery of the heavy isotope of hydrogen, a great number of "biological" experiments have been conducted with a variety of concentrations and with a diversity of complex phenomena such as growth, longevity, enzymatic reactions, oxygen consumption, luminescence, and permeability relations. For reviews of the current literature Barnes and Jahn (1934) or Fox (1934) may be referred to. In general, the work with low concentrations of deuterium shows a diversity of results; contradictions frequently occur as to the effects upon the same biological processes. A depressing effect has not usually been found. On the other hand, indications of stimulation or of the absence of effects exist.

In the work with Protozoa, a stimulating effect in respect to population numbers was reported with *Euglena gracilis* in D_2O of 1.000061 density by Barnes (1934). This would indicate that some life processes are speeded up in this weak concentration; Barnes and Larson (1934) also hold that the life span of primitive cells such as *Spirogyra* is increased by dilute solutions (e.g. 0.06 per cent) of deuterium. It appeared to have little or no effect upon the rate of cell division or upon the rate of elongation of the cells. The autodigestive processes of the planarian, *Phagocata gracilis*, during starvation were considerably slowed down in 0.06 per cent. With a concentration of 0.13 per cent to 0.47 per cent D_2O, the effects were "progressively obscured" due to the increase of bacteria and molds. Clearer results probably could have been obtained by a frequent change of the solutions.

Harvey (1934) as well as Taylor, Swingle, Eyring, and Frost (1933) reported no toxic effects with low concentration upon *Paramecium caudatum;* however, no actual account of fission rates or length of life was given.

Barnes (1935) also stated that "workers who claim that dilute heavy water has no influence on living organisms have not allowed sufficient time for the appearance of recognizable effects." It was with this very purpose that an analysis of the fission rate of the ciliate protozoan,

Uroleptus mobilis, during its complete life cycle was made. Since fission rate is one of the indications of the state of vitality of this protozoan, it was proposed to find whether a dilute concentration of deuterium oxide would alter this vitality over a long period of time, and if it would produce a stimulating effect such as has been reported upon the flagellate, *Euglena,* by Barnes. Secondly, it was proposed to note any changes in the total longevity of the culture. This type of isolation work involves no obscuring of effects by molds or bacteria since each animal is placed daily into fairly constant, fresh medium.

With intermediate concentrations, Taylor, Swingle, Eyring, and Frost (1933) reported that *Paramecium caudatum* may survive for about 72 hours in 60 per cent to 70 per cent D_2O, although many die after 48 hours. A concentration of about 50 per cent was selected to test the effects upon the fission rate of *Uroleptus mobilis* and its length of survival. The contractile vacuole rate was also observed as an additional method of noting any changes upon direct immersion into 50 per cent D_2O. The results will be compared below with those obtained by Barnes and Gaw (1935) for the immersion of *Paramecium caudatum* into 30 per cent D_2O.

The work with high concentrations has yielded relatively consistent results, namely that the protoplasm of all organisms cannot survive for more than short periods. Several Protozoa have been tested. Harvey (1934) found that *Amœba dubia,* as well as an unnamed species, was killed in 6 to 20 hours, and that although *Euglena proxima* exhibited avoiding reactions, then rounded up and became immobile soon after immersion in 90 per cent D_2O (a certain proportion remaining mobile, however, for three days), activity can be regained after five days by a return to normal water. With *Euglena gracilis,* recovery was possible after nine days in the D_2O, thus indicating that the animals were not "irreversibly injured." However, whether the treated animals would show delayed effects in their subsequent life history was not tested. Taylor, Swingle, Eyring, and Frost (1933) found a similar decrease in motility for *Euglena viridis* in 92 per cent D_2O, although recovery tests were not made. *Paramecium caudatum* swam slowly upon immersion into 97 per cent D_2O; in two hours the contractile vacuole enlarged with a cessation of contraction; blisters formed and it died within less than 24 hours (Harvey). Taylor, Swingle, Eyring, and Frost state that more time was required to kill *Paramecium caudatum* than frogs, fish, or flatworms, and they note that "*Paramecium* shows more resistance to heavy water than highly organized animals studied." If *Uroleptus mobilis* had been selected by chance instead of *Paramecium,* it would have been found that no more time for disintegration was needed, since

this ciliate usually collapses at once. This scattered work points to the fact that until a systematic immersion of various types of Protozoa is made to answer such questions as the correlation of the kinds of pellicles with the degree of resistance or whether animals in the encysted state may be more resistant to high concentrations, no general comparisons can be accurately made.

I wish to express my appreciation to Dr. Gary N. Calkins for the suggestion of this work and for his helpful advice, to Dr. James Curry for the making of the dilutions, to Dr. T. M. Sonneborn for his criticisms, and to Dr. H. S. Jennings for permission to complete the work at the Johns Hopkins University. The deuterium oxide was supplied by the Chemistry Department of Columbia University upon a grant from the Rockefeller Foundation.

Material and Methods

Cultures in 0.44 Per Cent Deuterium Oxide

The descendants from each member of two pairs of conjugating *Uroleptus mobilis* which were taken from stock mass cultures were selected for study. From each single ex-conjugant one series of descendants was carried in isolation cultures throughout the life cycle; these series will be designated as *A, B, C,* and *D* respectively. Series *A* and *B* are derived from the members of one of these conjugating pairs; *C* and *D* from the other. In each series there were six isolation lines, each line being one continuous sequence of descendants.

The medium for the normal control lines was made in the same way as that of Calkins for *Uroleptus* (1919); 50 mg. of timothy hay were boiled for 5 to 10 minutes in 50 cc. of Great Bear Spring water to which a few grains of white flour were also added. A second type of medium was prepared in the same manner, except that redistilled water was used instead of the usual spring water. For the medium of 0.46 per cent D_2O, a modification in preparation was made due to the sufficient but limited supply of the material. In order to produce a corresponding concentration of daily food supply in the 0.46 per cent D_2O, 50 cc. of distilled water with the same proportions of hay (i.e. 50 mg.) and flour as above were boiled to 10 cc. The same amount of hay was again added and the liquid was further boiled to 1 cc. and allowed to remain for 24 hours. The D_2O medium was then made up in the proportions of one drop of concentrated medium to 24 drops of 0.46 per cent D_2O; the concentration of D_2O was thus lowered to about 0.44 per cent D_2O. The 0.46 per cent D_2O came from the same lot as that used by Curry, Pratt, and Trelease (1935) (for preparation of which, see same).

All lines of each series were run for the first four ten-day periods in normal medium. During Period 5 in Series *B*, *C*, and *D*, and Period 6 in Series *A*, two lines of each series were transferred into the redistilled water medium and two lines into the 0.44 per cent D_2O by gradual changes extending over six days. For protection against evaporation and change of concentration of cultures, the depression dishes were fixed with vaseline covers. The cultures were continued in the same type of medium until the end. When one line died, a substitution of an animal from another line in the same type of medium was made.

The average fission rates for ten-day periods were calculated after Calkins (1919). If a line was ended by death of an animal during the ten-day period, it was not used in the calculation. If death occurred in all lines which were in the same medium at various times during a ten-day period, the average was made by averaging the fission rates of the preceding and succeeding periods (Calkins). Since the animals were under unfavorable conditions of transportation during Period 8, the values were also calculated in this way. For the first four periods, the six lines of one series were averaged together.

During Periods 1 to 4, the animals remained at room temperature which was around 24° C. An extreme temperature variation killed some of the animals in Period 4; the cultures were thereafter kept at a temperature of 24° to 28° C. Although this temperature variation doubtless accounts for some of the variation in the fission rate from period to period, it introduces no error into comparisons made synchronously, since all cultures were exposed to the same temperature at any one time.

Cultures in 48.1 Per Cent Deuterium Oxide

The descendants from one ex-conjugant of a conjugating pair which was selected from a stock culture of *Uroleptus mobilis* were carried in six lines for the controls. All lines which were started in the 50 per cent D_2O medium or in the special control water were derivatives from the control lines; in all cases, six lines of each type were carried. The normal and heavy water media were made up in the same way as those in the former experiment except that 50 per cent D_2O was used instead of 0.46 per cent D_2O.

The 50 per cent D_2O was prepared by the dilution of purified 95 per cent D_2O. For a part of the experiment, the animals were subjected to a special type of control water which was put through the same process as that used for the preparation of the deuterium of high concentrations, except that it had a concentration of deuterium which

corresponded to ordinary distilled water. After dilution with the necessary food solution, the 50 per cent water was about 48.1 per cent. From Period 10 on, distilled water was substituted for the spring water in the controls.

From Period 1 to 6, the temperature ranged from 24° C. to 28° C. During Periods 6 through 8, the animals were transported several times and remained at room temperature. After Period 8, they were kept at 22° to 27° C.

In order to test the abundance of the bacteriological food supply in the 48.1 per cent D_2O, four samples of the medium after 24 and 48 hours were plated upon nutrient agar which was made up with the 50 per cent D_2O, and were compared with four samples from the control lines made upon normal agar upon the same day during Period 25 and at the same temperature. As much care as possible was taken to plate approximately the same amounts of fluid by following one type of procedure, although this type of quantitative work cannot be accurate even at its best.

In twelve cases, the contractile vacuole rate of *Uroleptus mobilis* was observed before, after, and during immersion into 48.1 per cent D_2O. The specimens came from the isolation lines of the group of animals which was used as controls in testing the effects of 48.1 per cent D_2O upon the fission rate; they were taken at random, but all came from Period 19 in which the average fission rate was 13.1. Before observing, the animals were placed in several drops of fresh, normal medium for one to two hours. The animals were placed in one drop of the 48.1 per cent D_2O medium in a depression slide covered with a vaselined coverglass for observation. The D_2O medium was made up with nutrient in the same manner as in testing the fission rates. Upon immersion of the animal, the fluid was carefully mixed with the pipette. Upon removal, the animal was briefly washed through three or four drops of the normal medium, before observing. For any continued observation the animal was transferred to fresh medium every two hours. The figures used represent the averages of the number of seconds between successive contractions of the vacuole, based upon about ten consecutive measurements recorded by the use of two stop watches.

Results

Low Concentration

In mass cultures from all three types of media, i.e., the spring water, redistilled water, and the 0.44 per cent D_2O medium, normal and similar

amounts of conjugants as well as encystments were obtained during the life cycle. Thus 0.44 per cent D_2O has no striking effect, as revealed by this general method, upon the ability of animals to conjugate or encyst.

In considering the effect of the low concentration of 0.44 per cent D_2O upon a measurable function such as the fission rate of the ciliate, *Uroleptus mobilis,* no changes beyond the normal variation occurred, not only during a short time such as ten to twenty days, but also during a prolonged contact for at least ninety days with this low concentration

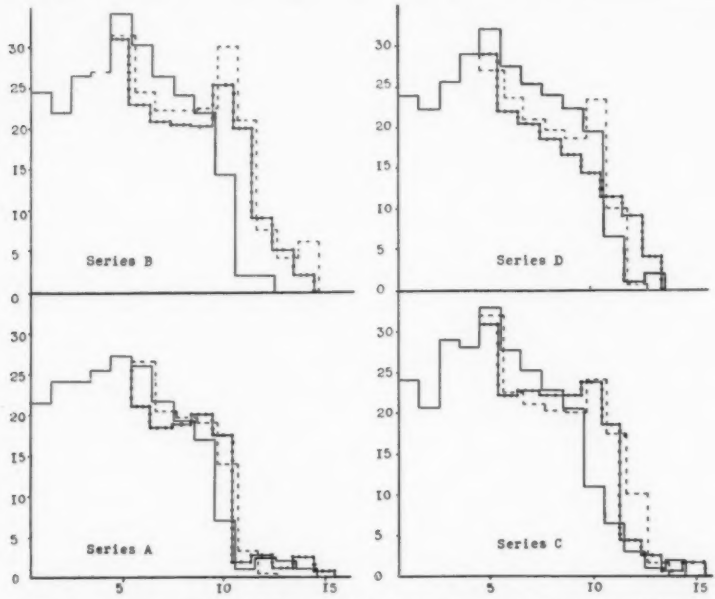

FIG. 1. Average number of fissions for four series: *A*, *B*, *C*, and *D*, during the life cycle in media made with spring water (——), redistilled water (– – –), and 0.44 per cent D_2O (–·–·–). Ten-day periods (abscissæ) are plotted against the average number of fissions for ten days (ordinates).

of D_2O. No accumulative or delayed effects were found, as may be seen from a comparison of the graphs for the four series in Fig. 1. For the most part, the 0.44 per cent D_2O lines followed those in redistilled water more closely than those in the normal control medium. Except for short divergences in these two media such as in Series *A*, Period 6; Series *B*, Periods 10 and 14; Series *C*, Period 12; and Series *D*, Periods 10 and 12; the rates were almost identical throughout the seventy or more days. In all media the appearance of the animals was normal.

Furthermore, in considering the total number of generations, it was found that the average number for the animals in 0.44 per cent D_2O cultures was intermediate in three series (*A*, *B*, and *C*). In Series *D*, the average of 233.5 generations in 0.44 per cent D_2O was slightly less than that of 242.5 generations for the redistilled water cultures or 254.5 for the normal controls. These differences in all cases lie within the limits of normal variation. In no series is there even a slight indication that the total longevity is prolonged or cut short by the contact of the animals with this low concentration of D_2O.

The percentage of animals surviving in the 0.44 per cent D_2O for seven periods after immersion was of the same magnitude as in the other two media for the same length of time. During this time, not

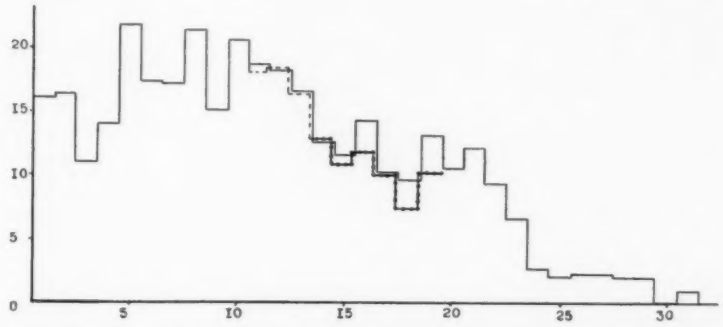

FIG. 2. Comparison of fission rate of Group *A* (——) in normal medium, and Group *B* (– – –) in special control water put through the same process as the 48.1 per cent D_2O. Return to normal medium ——. Abscissa = ten-day periods. Ordinate = mean number of fissions in ten days.

more than two deaths occurred in any one line; there was only one death in all four series during the transitional period in the 0.44 per cent D_2O lines.

Intermediate Concentration

Conjugation and encystment tests made on animals which were living in normal spring water or in special control water usually yielded many viable conjugants and cysts. In the greater proportion of cases in the 48.1 per cent D_2O, conjugation was not obtained since the conditions of the medium became toxic before enough animals could be obtained by their slow division to produce conjugating conditions. In the few cases where enough animals were obtained, conjugation always occurred in

good proportions but the ex-conjugants never survived. In two to three days, after conjugation in the culture, the ex-conjugants became small, either subsequently disappearing or forming what appeared to be cysts.

Attempts at ex-cystment were unsuccessful. Similar results for the ex-conjugants from 128 conjugating pairs which were isolated in fresh D_2O medium were obtained. These also could not be revived in 48.1 per cent D_2O and disappeared after a time. Twenty-four conjugating animals were placed in normal medium; 3 of the ex-conjugants died within ten days, but the other 21 survived. Their subsequent fission rates, however, were not tested.

The normal control animals will be designated as Group *A*; derivatives from them, as Group *B*, as shown in Fig. 2. The former group

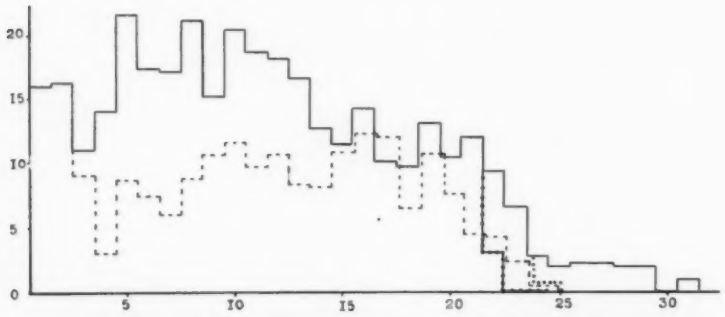

FIG. 3. Effects of 48.1 per cent D_2O on fission rate and length of life cycle of animals immersed in it at various ages. Group *A* (——), in spring water; Group *C* (– – –), *D* (— · —), and *E* (· · ·), in 48.1 per cent D_2O. Group *C* introduced into D_2O when mature, Groups *D* and *E* introduced into D_2O when old. Abscissa = ten-day periods. Ordinate = mean number of fissions in ten days.

survived for 31 ten-day periods and its fission rate was practically the same as that of Group *B*. This similarity existed both in Periods 11 to 13, when Group *B* was carried in special control water; and during the following six periods, when Group *B* was carried in normal medium. This shows that any changes found in the 48.1 per cent D_2O medium are not due to the presence of injurious agents, since this special control water was carried through the same process as the 95 per cent D_2O from which the 50 per cent D_2O was diluted.

At the end of Period 2, when the animals were in the forty-fifth to forty-eighth generations, derivatives (Group *C*) from each of the control lines were placed in 48.1 per cent D_2O, and allowed to remain until they died (Fig. 3). During the first fifty days (i.e., Periods 3 to 8)

the fission rate decreased to about half that of the normal animals so as to approximate that of old animals in a declining period such as No. 22 or 23. After a slight rise in Periods 8 to 10, the rate continued near the new level for about ninety days to Period 20. A decrease set in which ended in the death of the animals in about fifty more days. The slight increase in the rate may be interpreted as a delayed adjustment of the animals to the new environment, but due to the chemical or physical effects of the D_2O, the adjustment or return to the normal level was impossible. From Periods 15 to 20, the rates of both the control animals and those in 48.1 per cent D_2O were at the same level because the former were in the early stages of their decline.

It is also seen from Fig. 3 that although 48.1 per cent D_2O depressed the fission rate greatly during " maturity " (i.e., that time during the life cycle in which the animals are dividing at a high rate), the animals showed " aging " (the first signs of a general decline in the fission rate) at about the same time as the controls. However, " senescence " (period of very low fission rate which is followed by death) was cut much shorter.

Not only did the animals which were placed in 48.1 per cent D_2O during a period of high vitality divide more slowly but they also did not live as long. The animals began to die off in Periods 23 to 24, with an end to all lines in Period 25; while the controls did not start to die off until Periods 27 and 28, and ended in Period 31. Consequently, since the fission rate was lower in 48.1 per cent D_2O and since the length of life was shorter, the total number of generations was less, an average of 220.6 generations as compared to one of 366.1 for those in normal medium.

That these changes, in deuterium of this concentration, were not due to a continued insufficient diet can be assumed from the results of bacterial checks on the medium. In each case of the samples taken after 24 and 48 hours from the normal and heavy water cultures, the number of bacterial colonies upon nutrient agar was approximately the same. Also the same types of colonies in the same proportions appeared. Although no further examination of exact ratios was made, this roughly indicated that the bacterial food supply was at least ample in quantity and quality.

When normal old animals (Group *D*) in the 328th to 346th generations at the end of Period 21 were placed in 48.1 per cent D_2O, the fission rate fell to 3.0 and soon to zero (Fig. 3). By Period 25 all lines were dead, thus living only for a short time. Although these animals were dividing at a rate of 12.0 fissions per ten days just before immersion as compared with that of 16.3 fissions per ten days for the first

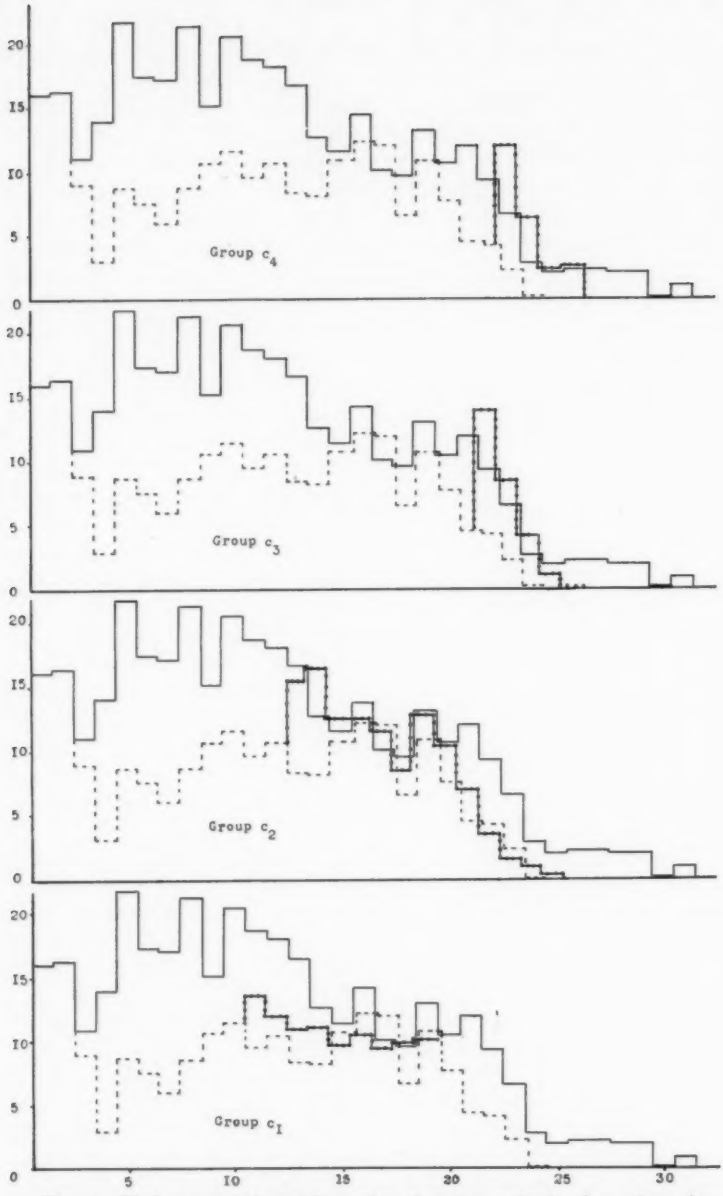

FIG. 4. Fission rate and total longevity of mature animals after release from long exposure to 48.1 per cent D_2O for various lengths of time. Normal controls, Group *A* (——); Group *C* in 48.1 per cent D_2O (– – –); Group c_1, c_2, c_3, and c_4 after return to normal medium (— — —). Abscissæ = ten-day periods. Ordinates = mean number of fissions in ten days.

group which was immersed after Period 2, it does not seem probable that this difference would account for the short survival time of Group *D*, but that the latter is due to the age of the animals upon immersion.

Similarly, when a third group (Group *E*) was placed in 48.1 per cent D_2O at the end of Period 23 (Fig. 3), the survival time was less than one period. The longest length of life of any individual was thirteen days while the average was five days for this group. Again after Period 27 when the normal group was very old, similar results were obtained.

Derivatives from the animals in 48.1 per cent D_2O were placed back into normal medium at five points, i.e., at the beginning of Periods 11, 13, 22, and 23 (Groups c_1, c_2, c_3, c_4, respectively). In each case the fission rate rose during the first period of this return (Fig. 4); except for Group c_2 it did not closely approximate the normal rate at the same time. In Groups c_3 and c_4 the rate rose to a level which was above that of the normal controls for the same period. Those in the first lot to be removed (c_1) were placed in the special control water which was later substituted by the normal medium after Period 13. The other groups were placed directly in normal medium. Since the first group was discontinued after Period 19, no further statements can be made concerning it. None of these released groups which were continued to the end of the life cycle lived as long as the normal control animals. Some were also removed at the end of Period 25; they died within a few days with no divisions, thus following the same course as the animals in the 48.1 per cent D_2O at that time. Since all of these groups died before the animals in the normal medium, they must have been slightly weakened by the long contact with the D_2O.

On the other hand, when derivatives (Group d_1) were removed at the end of Period 24 from the group of old animals which had been placed into 48.1 per cent D_2O from normal medium after Period 21 (Group *D*), they lived as long as the normal controls (Fig. 5). The release was accompanied by a stimulation which caused the fission rate to rise to 7.7 during the first ten days while the controls (Group *A*) were dividing at a rate of only 2.1. However, this high rate declined in the next sixty days so that the animals died at the same time as the normal controls. In spite of their age, the relatively short time of thirty days in D_2O had no permanent harmful effects, although the rate was strongly suppressed while in this medium.

For a secondary indication that the intermediate concentration changes life processes, a few measurements of the contractile vacuole rate were made. In transferring a normal *Uroleptus mobilis* to 48.1

per cent D_2O from the normal medium, there was an immediate decrease in the rate (i.e., within 15 minutes or less). Out of the twelve cases examined, there was one exception in which no regulation occurred and the animal suddenly disintegrated within two hours while still in the heavy water. Whether or not this lack of regulation was the cause of the death cannot be said. The general decrease was greater than any sudden fluctuations which occur in the normal rate. For six animals measured over a period of 4 to 5 hours with observations every 20 minutes in the normal medium, the greatest change was 3.9 seconds in 20 minutes; while upon transfer into 48.1 per cent D_2O, the decrease varies in extent from 6.1 seconds to 21 seconds for different animals. The majority fell between 7 and 15 seconds, although an in-

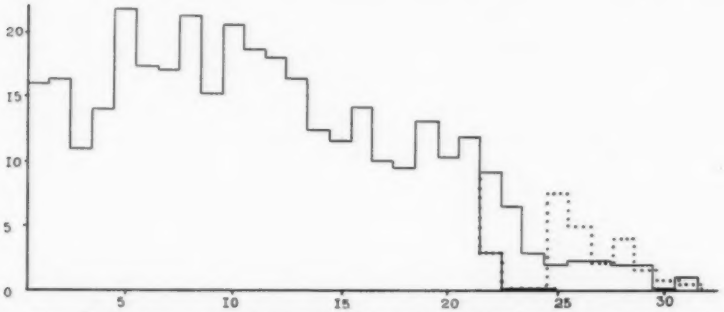

FIG. 5. Fission rate and total longevity of old animals after release from a short exposure to 48.1 per cent D_2O. Normal medium, Group *A* (——). Group *D*, animals put into D_2O when old (– – –). Group d_n, animals returned to normal medium after 30 days in D_2O (···). Abscissa = ten-day periods. Ordinate = mean number of fissions in ten days.

sufficient number were tested for averages. Consecutive immersions were not tried. Immediately upon immersion, in some cases, the contraction of the vacuole was very irregular, although within half an hour it became fairly constant. The degree of decrease did not depend upon the initial rate. Therefore each animal seemed to regulate itself according to its individual state at the time of immersion. The obtained variation in the decrease occurs within a temperature range of 2° C.

It is very difficult to assign one value to the rate while the animal is in any effective concentration of deuterium for a short time because the rate may fluctuate. The initial decrease may not be lasting or constant. In considering four animals which were kept in the D_2O from four to six hours, one of these animals regained and increased above its normal initial rate in less than three hours after immersion, although

its original decrease upon immersion was as great as that of the other three animals which continued to show a low rate in the D_2O until their release. A more extensive experiment would probably show more of this type. Also in the seven other cases examined over a much shorter period of time, there were two which gained the approximate original level in less than one hour and one which showed an increase in the rate just before it was released. Here also the initial change was as great as in the four other cases whose rates did not increase while in the D_2O. Whether any of these animals would have reached the original level after four to six hours cannot be told. There is also the possibility that these fluctuations are only preliminary and that over a longer period of time such as 24 hours a marked difference in the 48.1 per cent D_2O might show.

TABLE I

Differences in contractile vacuole rate between animals living in normal water (Group *A*) (Group c_1) and those in 48.1 per cent D_2O (Group *C*).

	Average contractile vacuole rate	Number of animals observed
	seconds	
Normal controls. Group *A*	19.7	13
In 48.1 per cent D_2O. Group *C*	28.9	27
After release from 48.1 per cent D_2O. Group c_1	20.6	8

This possibility is further substantiated by data obtained from measuring the contractile vacuole rates in samples of animals taken from Group *A* (normal medium) and Group *C* (48.1 per cent D_2O) during Periods 9–15 inclusive, as well as from Group c_1 (normal medium after eighty days exposure to 48.1 per cent D_2O) in Periods 12–15 inclusive. Table I shows that there is a clear distinction between the average rate of animals in normal medium and those in the heavy water; the animals which had been returned to normal water for ten days (Group c_1) had already gained the normal rate.

Upon removal from exposure to D_2O for a short time there was a corresponding change in the contractile vacuole rate which varied in its magnitude for various animals. In nine cases out of the eleven which were tested (above), the change was as great or greater than the highest variability while the animal was still in the deuterium, so that removal as well as immersion may cause a real change in the rate. Varia-

tion was present here also. In most cases the change restored the rate to or near the original level in half an hour or less.

High Concentration

When normal vegetative animals were immersed in 98 per cent D_2O an immediate quick shrinkage occurred, resulting in the formation of spherical or somewhat truncated shapes with the cilia still beating. Usually in five seconds to one minute these balls disintegrated. In a few cases the animals survived in the shrunken state for more than two hours. One animal which became about half of its original size during four minutes in 98 per cent D_2O gradually regained its normal appearance after transferal to normal medium and lived for thirty hours. Usually there was no recovery from these short exposures. Even although most specimens die at once, some distinct variability is apparent in *Uroleptus mobilis*.

Discussion

The facts indicate that neither the amount of conjugation or encystment in mass culture, nor its total longevity, nor the fission rate of *Uroleptus mobilis* during its life cycle are altered by a low concentration of deuterium and they are in harmony with indications from other work that low concentrations neither accelerate nor slow down certain life processes. On the other hand, these results are in contrast both to the stimulating effect upon the increase in population numbers of *Euglena gracilis* in mass cultures as reported by Barnes (1934), and to the increase of the life span of primitive cells such as *Spirogyra* (Barnes and Larson, 1934).

It is interesting to note that very young ex-conjugants from animals which have conjugated in the 48.1 per cent D_2O never survive if they are allowed to remain in this medium, but will live if the conjugants are transferred to normal hay infusion. Assuming from this latter fact that the conjugation process in the D_2O is regular, which should be checked as far as possible, the high susceptibility of conjugants and their young ex-conjugant derivatives is evident. Jollos has long maintained that conjugants as well as recent ex-conjugants are more sensitive to unfavorable conditions than animals during other stages. However, mature animals such as those which were placed in the 48.1 per cent D_2O twenty days after conjugation and subsequent fission to 45 to 48 generations in the normal medium, were more able to withstand the effects of this agent, although it is evident that their period of "senescence" was cut short. Furthermore, old animals, after 328 to

346 generations in the normal medium, have only a short survival in the deuterium. Although the discrepancy exists that the young animals conjugated in the D_2O and that the mature and old animals were derived from conjugation in normal hay infusion, these facts are in general accordance with the effects found by Gregory (1925, 1926) upon very young, mature and old *Urolepti* by continued treatment with beef extract or di-potassium phosphate, and by brief treatment with di-sodium phosphate, i.e., that both young and old series are immediately depressed while mature animals are stimulated in these media. Immature and old animals are not as able to adjust themselves to rapid changes in the environment. Thus these results not only show the actual effects obtained with an intermediate concentration of deuterium but also incidentally further substantiate the theory of Calkins (1926) that changes occur in the derived organization of *Uroleptus mobilis* throughout its life cycle.

That 48.1 per cent D_2O is clearly of a depressing nature is seen from the facts that both fission rate and total longevity are less in this medium. Also mature animals which have been in contact with this concentration for a long time, as from 90 to 230 days, are weakened. Although an immediate stimulation which may cause the fission rate to rise above that of the normal controls at the same time may occur upon release from heavy water, they do not live as long after they are returned to the normal medium as the control specimens which have never been exposed. On the other hand, even old animals are not weakened by shorter contacts as for 30 days, since they afterwards live as long as the controls; as above, the fission rate may even be temporarily stimulated upon release from heavy water to a rate above that of the normal controls. This clearly shows the necessity of carrying out work of this type with Protozoa over a long time in order to obtain the full results which may not be present at an earlier stage. This intermediate concentration, in contrast to the low concentration used, has a clearly depressing effect upon the protoplasm of *Uroleptus mobilis*.

The changes produced are not due to the lack of sufficient amounts or types of the bacterial food supply, nor are they due to any injurious impurity in the D_2O, since the fission rates of the animals in the special control water, which had been run through the same series of distillates as the D_2O, were equal to the normal rate.

With the few contractile vacuole rate measurements as a second way of testing the effects of an intermediate concentration, a slight decrease upon immersion is usually found. The great variation in the degree of decrease points to the fact that the evident attempts at regulation are

a function of the particular individuality of the protoplasm at this time. Each animal regulates itself according to its state, which is unknown to the observer. The decrease in contraction may continue within the first four to six hours, or it may regain the original rate which the vacuole possessed before immersion. However, it might reach a steady decreased rate or a complete recovery in all cases after a longer period such as twenty-four hours. That the former condition is the more likely is seen from the fact that animals which had been running in isolation cultures in the 48.1 per cent D_2O for sixty days were contracting at an average rate of 28.9 seconds as compared with that of 19.7 seconds for the control lines in normal medium. From the work of Barnes and Gaw on *Paramecium caudatum,* a decrease was reported after twenty-four hours immersion in 30 per cent D_2O. Finally, upon removal from D_2O, there may be a corresponding increase in the contractile vacuole rate which usually brings it back to or near its original level in those animals whose rate has remained lowered in the heavy water solution.

In working with the contractile vacuole rate of *Paramecium caudatum,* Barnes and Gaw (1935) state that "in a race of *Paramecium caudatum* the contractile vacuole empties every 18.9 seconds in 30 per cent D_2O and 11.3 seconds in controls at 18.8° C." They apply the Arrhenius equation to their data and find that in 30 per cent D_2O, the graph representing the log K as a function of $1/T$ is an unbroken line throughout the entire temperature range which was used, and that E (or the energy of activation of the controlling catalyst) equals 22,000 calories. Moreover, they found that in normal medium, the rate varies with the temperature, and from the rates assigned specific values to E at these temperature ranges. Since E below 16° C. was similar to the E in 30 per cent D_2O, they concluded that deuterium has similar effects to those of low temperatures.

In the first place, the extremes of 18.9 seconds and 11.3 seconds for contraction are within the limits of normal variation for *Paramecium caudatum.* The normal extremes can be far greater than the differences here stated by Barnes and Gaw. In studying the extremes of these variations, Pütter (1900), for example, found that in a total of 1,100 pulsations observed, the interval between pulsations varied from 6 seconds to 43 seconds; in the same animal, they varied as much as 21 seconds. Frisch (1935) further observed among other facts that swimming, for example, produces a marked decrease in the rate. The decrease begins suddenly when swimming commences and stops suddenly when it stops. Averages for several animals gave the results of 27.5

seconds at rest before swimming, 157.7 seconds swimming, and 28.1 seconds at rest after swimming. Furthermore, the rate varies when animals are at rest, depending upon the time of feeding.

Secondly, it is difficult to apply the Arrhenius equation to the contractile vacuole rate due to this variation. At the present time, we do not know the nature of the reaction or reactions which govern the contractile vacuole discharge. All that we can say is that the filling of the vacuole and its discharge may be the results of numerous types of reactions, of a possible catalytic nature; whether the same causative reactions are affected by deuterium and by temperature cannot be stated.

Finally Barnes and Gaw make no designations as to which of the two vacuoles in *Paramecium* were observed. Fortner (1924) as well as Frisch (1935) have noted that the posterior vacuole contracts more frequently than the anterior one, although in a few animals the reverse was true. Among a group of twenty animals, Frisch shows in his Table I (p. 13) that the variation in the difference of contraction may range from .2 second to 13.3 seconds in cases where the posterior vacuole is contracting the more rapidly.

Since so little is known at present about the function or regulation of the contractile vacuole as a typical but not universal character of the protozoan cell, it does not present itself as a favorable function for the basis of suitable experiments to ascertain the effects of D_2O.

In considering the effects of a high concentration, this protozoan, like other organisms, cannot survive for more than a very brief time in 98 per cent D_2O. In contrast to *Euglena gracilis* (Harvey) an irreversible injury is present.

Conclusions

1. No indications were found for the stimulation or depression of the fission rate of *Uroleptus mobilis* in 0.44 per cent D_2O during that part of the life cycle which covered about ninety days.

2. On the other hand, with a concentration of 48.1 per cent D_2O both the fission rate and the total longevity were decreased. The degree of the decrease depends to some extent upon the age of the animals since the previous conjugation. Young and old animals survive for only a short time such as thirty days after immersion into 48.1 per cent D_2O; while mature animals live for many days. This shows the necessity of knowing the history and of controlling the experimental material in such organisms as Protozoa. Short exposures to 48.1 per cent D_2O such as for thirty days even with old animals have no harmful effects, as evidenced by the fission rate and the length of life after

return to normal water, whereas long exposures such as eighty days with mature animals result in a decrease in the total longevity.

3. In general, a lowering of the contractile vacuole rate occurs in 48.1 per cent D_2O. The extent of the decrease varies from animal to animal. However, due to the small amount of knowledge upon the exact causative reactions which govern the contraction as well as the normal variation in the rate for different animals, the function is not favorable for study in this respect in this protozoan.

4. Ninety-eight per cent D_2O causes an irreversible injury resulting in immediate death, although there is evidence that some few animals can withstand the effects for a few hours longer than the majority.

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THE STRUCTURE AND FUNCTION OF THE GUT IN SURF PERCHES (EMBIOTOCIDÆ) WITH REFERENCE TO THEIR CAROTENOID METABOLISM [1]

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In examining freshly-caught surf perches (Embiotocidæ) for trematodes, one of us (Young) encountered an interesting yellow or orange pigmentation of the gut, especially in the rectal segment of these fishes. Chemical and spectroscopic studies revealed the carotenoid (specifically xanthophyllic) character of much of this pigment. In freshly-caught specimens of these fish and of the spot-fin croaker (*Roncador stearnsi*) the rectum is frequently but not always deeply pigmented throughout its length to within a few millimeters from the anal opening, ranging in color from a brilliant orange to a deep brown. The upper intestine may also be colored to some extent, but it is the rectum which is most striking.

This observation suggested to us an experimental study of the rôle of the gut in the metabolism of these pigments, and for this purpose we selected *Cymatogaster aggregatus*,[2] a small perch which occurs abundantly in San Diego Bay and elsewhere along the west coast of North America. It has an average weight of 15–20 grams.[3] It is silvery in color with yellow markings on the sides of the body, which alternate with dusky bars.

The description of the gut which follows is based partly on that of *Cymatogaster* and partly on that of *Embiotoca jacksoni* and *Abeona minima*. The structure being very similar in all, the description of one will serve equally well for that of the others.

Several of the standard fixatives were used for the histological work including Bouin, Zenker, Fleming and Carnoy; while paraffin sections

[1] In this work, Young was responsible for the descriptive and histological portions, while the chemical analyses and spectroscopic observations were made by Fox. The experimental work was carried out jointly.

[2] A number of observations and experiments have also been made on other species, but *Cymatogaster* was the fish mainly used, and the one selected for all the quantitative studies reported here.

[3] Jordan and Evermann give its length as 6 inches but this is much above the average of our specimens, which is about 8–10 cm.

were stained mainly in Delafield and Haidenhain's hæmatoxylin, counterstained with eosin, and a few in Giemsa, as recommended by Lee.[4] With the latter stain we obtained some very fine results, especially of the mast cells and of the ganglion cells in the myenteric plexus. Besides studying the vascular supply of the gut in fresh material, we also injected two specimens of *Embiotoca* with India ink, one of which gave a very successful result.

The general anatomy of the digestive tract in surf perches is shown in Fig. 1. The bile duct enters the stomach just behind the pharynx, so that a true stomach is wanting. The rectum forms about one-tenth of the total length of the intestine, and is demarcated from the rest of the latter by a distinct sphincter.

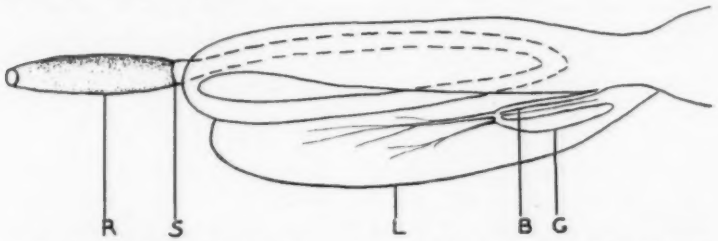
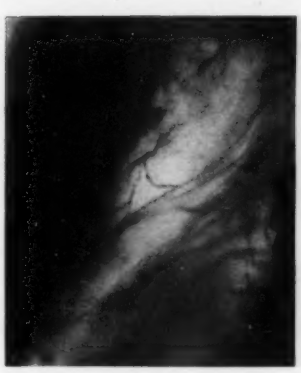


FIG. 1. Digestive tract of *Abeona* × 4. *B*, bile duct; *G*, gall bladder; *L*, liver; *R*, rectum; *S*, sphincter.

Internally the lining of the gut is similar throughout. It is elevated in narrow folds or ridges that follow an irregular, zig-zag course and in general run parallel to the walls of the gut, but whose form and direction is largely determined by the condition of the organ itself. If the latter is filled with food, the folds flatten out and assume a more transverse direction; if the gut is empty the folds heighten and follow more nearly a longitudinal course. At the apices of the angles formed by their bends the folds frequently send out offshoots which may end blindly, or occasionally anastomose with neighboring ridges (Fig. 4).

The blood vessels of the gut have been studied mainly in *Embiotoca jacksoni* instead of *Cymatogaster*, because of the greater ease of injecting them in the larger fish. After penetrating the two muscular layers of the gut wall the vessels branch frequently to form a plexus at the base of the mucosa (Fig. 2) whence smaller twigs extend through the latter to terminate in a very fine plexus at the summit of the folds, just below the epithelium (Fig. 3). In sections prepared from a specimen of *Embiotoca* injected with India ink, these twigs can be followed in

[4] Vade Mecum, Ed. 9, p. 503.



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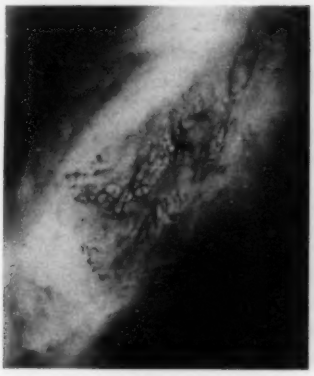

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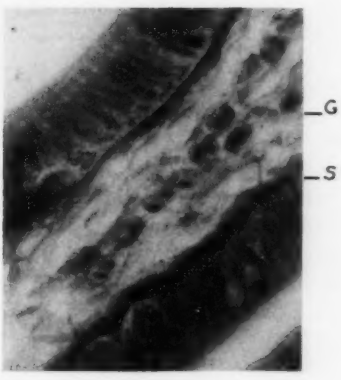

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FIG. 2. External vascular plexus from an injected specimen of *Embiotoca*.

FIG. 3. Internal plexus of same.

FIG. 4. Intestinal rugæ of *Cymatogaster*, from a tangential section. The stratum granulosum is the loose layer between the epithelial folds, while the compactum is the darkly stained line at their base. An anastomosis between two rugæ is shown at *X*. Three trematodes are seen in the lumen between the rugæ. × 130.

FIG. 5. Section of a ruga from the rectum of *Cymatogaster*. *S*, stratum compactum; *G*, stratum granulosum with mast cells. × 560.

sections close to the epithelium but careful search fails to reveal any penetration of the latter by the capillaries. There are several accounts in the literature, however, of the penetration of the epithelium by capillaries in the gut of the Cobitidæ.[5]

The wall of the gut is similarly constituted throughout, so that it is very difficult to tell what region any section represents. The absence of a muscularis mucosæ removes any distinction between mucosa and submucosa, regarding which considerable confusion exists in the literature (see Blake, 1930, and Rogick, 1931).

Greene (1912) divides this layer (mucosa + sub-mucosa) in the intestine of the salmon, where a muscularis mucosæ is absent, in contradistinction to the stomach where it occurs, into three sub-layers: tunica propria, stratum compactum and stratum granulosum. Both of the former authors describe a basement membrane of the epithelium which (see Blake, *loc. cit.*, p. 53) is formed from the tunica propria in the stomach of *Centropristes*.

In our own material basement membrane and stratum compactum are indistinguishable, the latter being well developed and sharply set off from the rest of the connective tissue, or stratum granulosum (Fig. 5). This is so named by Greene because of the presence within it of large numbers of granular cells whose " special and characteristic feature . . . is the presence of the cytoplasmic granules that always crowd them, uniformly filling the cell body " (*loc. cit.*, p. 83). In the salmon these cells are not restricted to the stratum granulosum, but occur also in the stratum compactum, and occasionally in the tunica propria and the sub-mucosa. Greene suggests that these cells form an internal secretion, probably lipase, which is discharged " directly into the surrounding tissue spaces from which distribution takes place " (*loc. cit.*, p. 84).

These cells have been described in detail by Bolton (1933) as mast cells whose " true function seems as yet to be as obscure as that of the mast cell of the higher vertebrates . . . (but which) may play some rôle in connection with the elaboration of zymogen in these organs " (*loc. cit.*, pp. 576–7).

In our material, cells which are undoubtedly the same as those described by these authors occur in considerable numbers scattered through the connective tissue of the mucosa. In some places they occur in groups, in others as isolated cells. They are most abundant in the rectum, but are widely distributed throughout the gut. In places the granules are scattered through the connective tissue, as though set free by the rupture of the cells, but for the most part, the cells are discrete, varying in shape from globular or ovoid to ameboid. Whether they

[5] See especially in this connection Abolin (1924).

have any relation to metabolism of the carotenoids in the gut, we cannot say.

The carotenoids are found in the mucosa of the gut, as is easily demonstrated by washing this tissue with a fine jet of water applied with sufficient force to remove it in patches, leaving it intact elsewhere. Where the latter has been removed the pigment is absent, but is present elsewhere. And when the mucosa begins to break down post mortem the pigment is readily washed out with it. Whether the pigment is restricted to the epithelium, or is present in the connective tissue also we do not know. However, when a brightly pigmented rectum is dissected so as to expose the mucosal folds, the pigment appears as lines or streaks in the latter, and since these are composed mainly of epithelium it is probable that this is the chief, if not the only locus of the pigments.

The source of the pigment is the food. The diet of the surf perches is a varied one, consisting of mussels, clams, barnacles, worms, many kinds of Crustacea and even fish. *Cymatogaster,* on which our experiments were mainly performed, feeds extensively on little green and brown shrimps (*Hippolyte californiensis*) [6] together with a species of *Crangon,* copepods, etc. When brought into the laboratory from the bay the recta are characteristically bright orange in color, while the upper intestine is salmon colored, fading out posteriorly, however, so that the last half centimeter or so is usually colorless, or very pale. The color of the upper intestine is more or less affected by the rich vascular supply of this region, so that in a faintly pigmented gut it is not always easy to determine how much of the color is due to carotenoid and how much to blood pigments. In other surf perches (*Embiotoca,* and *Hyperprosopon*) taken from the surf, and in specimens of *Abeona* which have been kept in aquaria and fed upon various diets, or left unfed, the color of the rectum varies from yellow, through orange to olive or brownish, depending upon the kinds of pigment, derived from food or other sources, or possibly upon chemical changes which the pigments may have undergone.

The rectal pigments which depart in color from pure yellows or orange are not carotenoids, as was demonstrated on several occasions by treating the ground tissue directly with lipoid solvents which failed to extract pigment. Water, or alcohol extracts assumed a pale cloudy yellow tint, but no pigment could be transferred from these solutions to ligroin (Eastman Kodak Co., B.R. 70–90° C.).

By no means all of the available carotenoid pigment in the food is

[6] The authors acknowledge their thanks to Dr. Martin W. Johnson, of the Scripps Institution of Oceanography, for verifying the identity of this shrimp.

absorbed, for the partly digested food and the feces of fish which have been fed shrimps are always colored, and brightly colored bits of Garibaldi tissue may be found in the feces of fish which have been feeding thereon. Even in a fish whose intestine exhibits no appreciable color the food may retain much of its pigment, as was shown by an *Abeona* whose gut was practically colorless, but which contained the brightly colored remains of a shrimp. Carotenoids may be recovered in considerable quantity from the feces of perches which consume shrimp and other crustacean food. (See below.)

Qualitative and Quantitative Studies of the Yellow Pigments

Several chemical analyses and spectroscopic observations [7] were made of the carotenoid pigment or pigments in each of the following: the entire intestines or the recta alone, the gut contents and feces, the shrimps upon which the perch feed, and the fish bodies minus viscera.

Rectal Segments

A number of these were removed from several specimens (some freshly caught and others having been fed in the aquarium for some days their natural diet of shrimps) and analyzed.[8] Trituration with a little sand and absolute methyl alcohol caused this orange colored tissue to yield its carotenoid material promptly and completely to the solvent, rendering it yellow orange in color. The pigment remained hypophasic, i.e., dissolved entirely in the lower aqueous-alcohol layer when shaken with mixtures of 90 per cent CH_3OH and ligroin, whether the alcoholic solution had or had not first been hydrolyzed for some hours in warm alcoholic KOH. Acidification or alkalization did not alter this characteristic. The pigment was easily transferred from the alcoholic solution (neutralized to preclude the formation of stubborn emulsions if necessary) to ligroin by adding sufficient water and shaking the mixture. It was quantitatively removable from alcohol-free ligroin solutions by adsorption on a column of dry, powdered $CaCO_3$, upon which it exhibited a pale yellow-red band at the top of the column, with a rose-colored band just beneath.

[7] Spectroscopic observations in this work were made with the large Bausch and Lomb spectrometer No. 2700. Absorption maxima were estimated, and the average of a number of readings taken, in each determination, since the accessory spectrophotometric equipment for securing more precise data was not at hand at the time that this work was done.

[8] In some analyses the whole intestines, from esophagus to anus, were used; in others, the rectal segments only. Often the intestine exclusive of the rectum appeared quite pale and yielded little or no pigment. Even when the absorbent portion of the gut was distinctly pigmented, no differences were detected in the nature of the extracted carotenoid material.

The carbon disulphide solution of the unseparated pigments was deep red orange in color, and exhibited absorption bands whose maxima averaged approximately: I 499.4 mμ, II 471.6 mμ.

Ethereal solutions of the pigments shaken with concentrated HCl remained colorless.

The foregoing properties of the pigment in the intestinal tissue (especially concentrated in the rectum) indicate that it is a carotenoid, or more probably a mixture of two carotenoids judging from the two rings in the $CaCO_3$ column, of a xanthophyllic nature, and unesterified. The absorption spectrum corresponds rather closely to those of taraxanthin and violaxanthin (Zechmeister, 1934; Lederer, 1935) of the $C_{40}H_{56}O_4$ series; the additional fact that concentrated HCl imparted no blue color to ethereal solutions of the pigment indicates that the preponderant carotenoid is not violaxanthin but probably chiefly taraxanthin or a carotenoid indistinguishable from the latter by these various tests.

No attempt was made to separate the adsorbed pigments in the chromatogram, since the total quantity of available pigment was very small, and the paleness of the primary ring indicated a relatively small quantity of this fraction. Furthermore, the spectroscopic picture of the rectal pigment was nearly identical with that of the shrimp xanthophyll, that of the fish skin xanthophyll, and with the body xanthophylls of other species of fish (see Fox, 1936). Why the rectal and shrimp xanthophyll chromatogram showed a red component, while the skin xanthophyll (hydrolyzed) chromatogram consisted in a yellow ring is yet unexplained, but it is possible that the xanthophyll stored in skin is either altered chemically without materially shifting the absorption bands, or rigidly separated from another carotenoid having a very similar spectrum.

Intestinal Contents; Feces

No differences were detected in the nature of the carotenoids of the ingested food removed from the gut and those found in voided feces. Absolute methyl alcohol extracted quantitatively all of the yellow colored fat-soluble compounds. Shaking a 90 per cent CH_3OH solution of the pigments with an equal quantity of ligroin resulted in most of the colored compounds remaining in the lower (alcoholic) layer, with some pigment being transferred to the ligroin phase above. Selective solubility in ligroin is characteristic of carotenes, esterified xanthophylls, free (neutral or acidic) astacene, and certain astacene esters. (See Zechmeister, 1934; Lederer, 1935; Fox, 1936.) That no free astacene was present in the ligroin was demonstrated by adding a drop of strong lye to the aqueous alcohol layer which did not, upon shaking, remove any

pigment from the ligroin phase. A portion of the whole extract was hydrolyzed in warm alcoholic KOH, usually for some hours, after which three components were easily separated, whose descriptions follow.

(1) A red substance, insoluble in ligroin or absolute CH_3OH, remained in flocks on the bottom of the flask. When collected on filter paper and washed several times with alcohol and ligroin the material was finely divided and bright red in color, resembling that of ripe tomatoes or red peppers. It remained completely insoluble in neutral ligroin, CH_3OH, or CS_2 but was readily dissolved in any of these solvents if acidified by adding a trace of glacial acetic acid. It exhibited, therefore, the properties of a fat-soluble acidic compound capable of forming a potassium salt which was insoluble in the various organic solvents. The acidified product yielded bright yellow ligroin solutions, salmon-colored CH_3OH solutions, and a beautiful rose pink to magenta color in CS_2. It was hypophasic when shaken with equal quantities of ligroin and 90 per cent CH_3OH. Dilution of the alcohol layer caused the free pigment to be transferred to the ligroin phase. Solutions in ligroin or CS_2 (always freed from the last trace of alcohol and acetic acid) when passed through a $CaCO_3$ column left the pigment quantitatively adsorbed to this salt in a single rose-colored ring at the top of the column. It was readily desorbed, as are the xanthophylls, by ligroin containing a trace of CH_3OH. This pigment was even adsorbed to some extent by pure, dry, crystalline NaCl imparting to this salt a delicate rose tint. It was desorbed by wetting the salt with a little water.

The pigment proved to be rather unstable chemically; when recovered from solvent and stored dry in a partially evacuated chamber, the red color faded completely in a few days. The deep red solution of this rather unusual, acidic carotenoid in CS_2 solution gave a very poorly-defined absorption shadow down in the violet, the average maximum absorption region, taken from numerous readings, lying close to 499.5 $m\mu$, which is somewhat lower than that of astacene (510 $m\mu$ in CS_2 according to Lederer, *op. cit.*). While also acidic, this compound differs markedly from astacene in other ways as well as in its absorption maximum, viz.: its preferential solubility in alcohol when subjected to partition between alcohol-ligroin mixtures; the insolubility of its potassium salt in alcohol (astacene, preferentially soluble in ligroin, can be transferred immediately to the alcohol layer by adding a drop of lye and reshaking (Zechmeister, *op. cit.*)), its instability in air, and its quantitative adsorption from ligroin or CS_2 solutions by $CaCO_3$. Were it not for the acidic character of this red carotenoid, it would correspond rather closely to Lederer's glycymerine, which is neutral, shows a single

absorption maximum at 495 mμ in CS_2, preferential solubility in 90 per cent CH_3OH, adsorbability on $CaCO_3$, and other characteristics which liken it to the xanthophyll group of pigments. Until further study may provide more information concerning this interesting pigment, no provisional name will be assigned to it.

(2) When the filtrate from the crop of red flocks was shaken with ligroin, a relatively small quantity of yellow pigment remained in the ligroin layer and was not removable therefrom by subsequent washes with 90 per cent CH_3OH, whether neutral, acidified, or alkalized.

The ligroin solution was freed from alcohol and acetic acid with repeated water washes and then tested chromatographically by passing through a $CaCO_3$ column. No pigment whatever was adsorbed. Transferred to CS_2, after evaporating the ligroin in a current of illuminating gas (to prevent bleaching from oxidation), the solution had an orange color, and exhibited absorption bands whose average maxima lay respectively at: I 511.7 mμ; II 480.9 mμ, which values correspond closely to the first two principal maxima in the visible region for CS_2 solutions of α-carotene (i.e., I 510 mμ; II 478 mμ) and depart somewhat from those of β-carotene (I 521 mμ; II 485 mμ).[9] That it is a carotene is shown by its neutral character, its preferential solubility in ligroin instead of in 90 per cent CH_3OH, and its non-adsorbability on $CaCO_3$. Its absorption spectrum would indicate that the preponderant part of it was closely allied to, if not identical with α-carotene.

(3) The hypophasic (alcohol-soluble) portion of the original filtrate of the hydrolyzed mixture yielded no further pigment to washes of ligroin applied subsequently to the first separation. After transference to ligroin by diluting the alcohol phase, shaking, separating, and finally washing the ligroin solution free from all traces of alcohol, the pigment was quantitatively adsorbed by $CaCO_3$ most frequently as a single yellow band (sometimes as a reddish band) at the top of the column.

The orange colored CS_2 solution of such pigment fractions gave absorption bands with average maxima readings at the following points: I 496.9 mμ; II 467.4 mμ. In its neutral character, hypophasic property, and its quantitative adsorption upon $CaCO_3$, this pigment, like that of the intestine, showed xanthophyllic properties. Also, its absorption spectral bands showed maxima which agreed rather closely with those of the intestinal pigment although the latter's maximal readings were placed slightly more toward the red. While the intestinal pigment always showed two rings, i.e., a yellow, and a red one just below it in the $CaCO_3$ chromatogram, the xanthophyll fraction of the feces

[9] These figures are for visual spectroscopic readings, but see Smith (1936) regarding photoelectric spectrophotometric methods which give shifts in the positions of absorption bands.

and food material in the gut did not always show two such rings, but frequently only the yellow one. The presence of the red ring as well as the yellow, indicating another compound, very possibly depended upon the stage of digestion and relative degree of absorption of the compounds which may have already occurred by the time that the feces or intestinal contents were collected. Since this feature in the chromatogram characterized the xanthophyll of both the intestinal tissue of the fish and the whole shrimps which formed its raw food, and hence the source of the carotenoids, one might have expected to find some such inconsistency in the relative amounts of the xanthophylls in the corresponding fraction of the food boli or feces. The red component in the chromatogram might have been either another xanthophyll or an incompletely hydrolyzed ester, since the esters were shown to be less readily sorbed by $CaCO_3$ and to show various color variations between yellow and red in a chromatogram.

Shrimps

These were gathered from the same locality as that from which the perch were taken, constituted the natural diet of the latter, and were taken from the lot which were fed the experimental animals whose tissues were subsequently analyzed. Two or more species were represented, but the predominant form was *Hippolyte californiensis*. Some two dozen of them were dropped into pure CH_3OH, whereupon they quickly lost their original color, whether brown, green, or dull red, and exhibited bright red patches upon various parts of their bodies. They were ground in a mortar with sand, and yielded all their carotenoid pigments to the alcohol.

All pigment was quantitatively epiphasic, being readily extracted from the alcohol by ligroin; shaking the ligroin solution of carotenoids with additional 90 per cent methanol did not cause any transfer of pigment back to the latter solvent.

After hydrolysis for some hours in warm alcoholic KOH, the shrimp pigments were, as in the extracts of the intestinal contents or feces, resolvable into three components, which were virtually identical in chemical and spectroscopic properties with the respective components of the latter, as follows:

(1) A red, acidic carotenoid, whose potassium salt was insoluble in alcohol, ligroin, or CS_2, but which when free was readily soluble in any of these; hypophasic property in ligroin + 90 per cent methanol systems; quantitative adsorption by $CaCO_3$ as a rose-red ring at top of column; poorly defined absorption band with maximum at approximately 494.5 mμ.

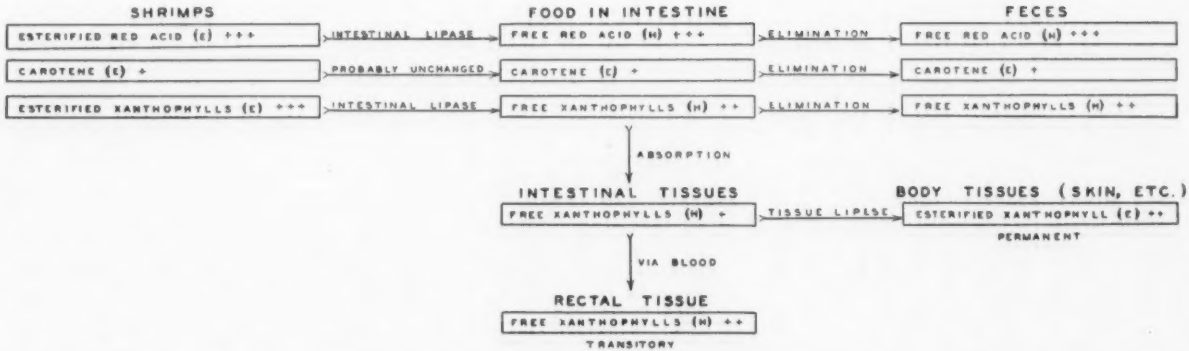

FIG. 6. The fate of shrimp carotenoids ingested by the surf perch *Cymatogaster aggregatus*. (*E*) denotes that the pigment is *epiphasic* and, excepting in the case of carotene, esterified; (*H*) denotes that the pigment, having been hydrolyzed, is *hypophasic*. The crosses represent, in the conventional manner, not absolute quantities but merely approximate relative concentrations of pigments (D. L. Fox and R. T. Young (Drawn by C. W. Watson)).

(2) A small quantity of an epiphasic carotenoid, unadsorbed by $CaCO_3$, and with absorption bands at approximately, I 513.1 mμ; II 477.7 mμ.

(3) A hypophasic xanthophyll component quantitatively adsorbed by $CaCO_3$ in a yellow ring nearly at the top, and a faint rose ring just beneath it. The reddish orange CS_2 solution of this pigment gave absorption bands with maxima at the approximate average positions: I 500 mμ; II 469.1 mμ; which agree rather closely with the respective absorption bands of the xanthophyllic pigment extracted from the gut tissue and with the corresponding component of the shrimp tissue in gut or feces. Like these pigments, also, this one from the shrimps gave no color reaction with concentrated HCl.

It is important to note that while the xanthophyllic pigments of the gut contents or feces of the fish, as well as those of the intestinal and rectal mucosa were free (i.e., unesterified, hence preferentially soluble in 90 per cent CH_3OH), the corresponding components in the shrimps which formed the original food and from which the fish pigment was derived were completely esterified, manifesting this property by complete preferential solubility in ligroin. Similarly, the red acidic carotenoid must have undergone hydrolysis in the gut, through the agency of lipase, for, whereas in food boli from the gut or in fecal material this pigmentary constituent was completely hypophasic as were the xanthophylls, the same compound was completely epiphasic in the original living shrimp tissue. It is possible that in nature the red acidic carotenoid may be conjugated with the alcoholic xanthophylls.

The behavior of this red acidic carotenoid recalls that of astacene, which may exist, according to Kuhn and Lederer (see Lederer, 1935; Zechmeister, 1934) as epiphasic esters, preferentially soluble in petroleum ether, or as hypophasic esters, more soluble in 90 per cent CH_3OH, in different tissues of the Norwegian lobster.

Fish Bodies, Exclusive of Viscera

Bodies of whole eviscerated perch which had been fed shrimps for some days in the aquarium were chopped up with scissors and then ground with alcohol in a bronze ball mill (Sumner and Fox, 1935*a*) until the material was reduced to a fine pasty consistency, the proteins being denatured and relatively dehydrated by the alcohol, and the carotenoid rendered subsequently easily extractable. The most striking observation was that the pigment was hardly extracted at all by methyl alcohol at this stage, but was quantitatively removed by ligroin. Needless to add, it was completely epiphasic. Also interesting was the observation that the xanthophyll ester (see below) seemed to be more

resistant to hydrolysis than were those which occurred in the original shrimps consumed by the fish. This may have been due either to different fatty acids having been involved in the esterification, or to more hydroxyl groups having been esterified in the fish body pigment, for the xanthophyll itself was by all tests identical with the principal one found in the food and rectum.

The spectrum of the epiphasic, unhydrolyzed compound in CS_2 showed two strong absorption bands with average maxima at I 497 mμ; II 468.6 mμ.

After hydrolysis in alcoholic KOH, the pigment was quantitatively hypophasic, and was completely adsorbed from ligroin solutions by $CaCO_3$ as a yellow layer at the top of the column. The orange-colored CS_2 solution showed absorption bands with average maxima at I 497.5 mμ; II 466.8 mμ. It will be noted that the xanthophyll, apparently a single compound, in the tissues (especially skin and fins) of the perch showed, after hydrolysis, the same chemical properties as did the xanthophylls from the gut and rectum and the xanthophyll fraction from the shrimps, except that the latter two pigments showed a secondary reddish adsorption ring in the $CaCO_3$ chromatogram. The two respective spectral absorption maxima of the xanthophyll pigment from all three sources are in close agreement; confirming the conclusion that we are dealing with a single carotenoid as the sole pigment in the skin and other non-visceral tissues of the fish, and as the certainly preponderant xanthophyll in the rectum and in the original shrimp tissue.

Having determined the nature of the xanthophyllic pigments in the shrimps taken from natural surroundings, the same while undergoing digestion in the intestine of the perch, those in the rectal segment, and the eviscerated whole body of the perch, the same question as initiated the investigation still confronted us: what if any is the function of the rectal segment with regard to the metabolism of the carotenoid, and what is the probable fate of the carotenoid which is found temporarily stored in this structure?

Several feeding experiments were performed in the laboratory with *Embiotoca, Cymatogaster* and *Abeona,* using shrimps (mainly *Hippolyte*) sand crabs (*Emerita analoga*) and minced flesh of the Garibaldi (*Hypsypops rubicunda*) as food. The latter, as has recently been shown (Sumner and Fox, 1935*a, b;* Fox, 1936), abounds in xanthophyll. (Chemical and spectroscopic data indicate, furthermore, that the xanthophyll of *Cymatogaster* skin is identical with that of the brilliant Garibaldi, although far less copious).

These experiments were undertaken in order to trace the pigment in the gut of the fish. A detailed account of all of them would be not

only wearisome, but somewhat confusing, since individual fish differed rather widely in their response to this feeding. Suffice it to say that in general the pigment appeared in the intestine, with exception of the last half centimeter or so, shortly after feeding, to disappear within a day or two after feeding was ended. The recta did not take up the carotenoid pigment extensively in many of these experiments, and it is difficult to decide whether the color which was observed in them was due to the experimental feeding or to residual pigments of catabolic origin, or from the food consumed in nature. In one experiment an *Embiotoca* which had been fed *Emerita* on two occasions and was killed five days after the first feeding, and one day after the second, contained almost no color in the rectum, while in another experiment a *Cymatogaster*, which had been fed shrimps for several days previously had no apparent color in the rectum. Occasionally, however, the recta were brightly colored in fish which had been previously fed on carotenoid-rich food.

While the intestine proper is undoubtedly a digestive and absorptive organ, secreting enzymes which hydrolyze both the esterified xanthophyll and the combined red, acidic carotenoid, subsequently selectively absorbing the free xanthophyll, the possible function of the rectum with regard to the carotenoid is still uncertain. It may be an organ for (1) absorption, although this possibility seems very doubtful, (2) storage, (3) elimination, (4) destruction (oxidation?) of the pigment, or some combination of these.

It was of interest to determine whether or not the rectum might be a kind of storage organ for xanthophyll for the re-supply of other body tissues which might utilize it. Accordingly, a group of *Cymatogaster* from San Diego Bay were held in a laboratory aquarium for some weeks, several of their number being sacrificed at stated intervals of days, eviscerated, weighed, and the body carotenoid quantitatively extracted and determined in the regular way. At the same time the whole gut was carefully examined and its appearance with regard to pigmentation recorded. The quantities of rectal xanthophyll were analyzed for the first two lots of fish killed, but in later examinations the amounts of carotenoid in rectal tissue were so small that quantitative determinations were impossible.

The perch refused proffered food, and therefore consumed nothing except a few minute copepods which they obtained from the sea water, from the beginning (January 14) to the end (February 18) of the experiment.

The ligroin solution of extracted pigment was, as in previous work (Sumner and Fox, 1933) brought in each case to a volume equal (in

TABLE I

Date killed	Serial Number	Days kept	Number and sex of fish	Recta	Weight in grams	Photometer reading	Xanthophyll (mg. per 100 gr. tissue*)
Jan. 15.....	C_1	1	4 ♀	Bright yellow orange	58.3	60.1	0.27
Jan. 15.....	C_2	1	2 ♀	" " "	29.6	60.2	0.27
Jan. 15.....	C_3	1	2 ♂	" " "	27.5	59.8	0.27
Jan. 14.....	C_4 (stored in refrig. until Jan. 17)	0	3 ♀	" " "	54.4	61.0	0.26
Jan. 18.....	C_5	4	4 } 5 ♀, 3 ♂	Paler and variable	59.8	61.5	0.26
Jan. 18.....	C_6	4	4 }	" " "	60.2	58.6	0.29
Jan. 24.....	C_7	10	3 (2 ♀, 1 ♂)	Pale, one brown	50.4	56.2	0.30
Jan. 31.....	C_8	17	4 (3 ♀, 1 ♂)	Pale	68.7	53.0	0.34
Feb. 8.....	C_9	25	1 ♀ (large, gravid)	Pale	45.1	64.7	0.22†
Feb. 18.....	C_{10}	35	2 (very small)	Pale	7.2	53.8	0.33
Jan. 15.....	C_1, C_2, C_3		8 recta		(0.4) ‡	62.4	2.5
Jan. 18.....	C_5, C_6		8 recta		—— §	81.4 (far paler)	1.0

* Calculated on a basis of the standardization of the Ives photometer with sunflower xanthophyll (largely lutein, provided by Dr. J. H. C. Smith and Dr. H. H. Strain of the Carnegie Institution of Washington, Stanford University). Xanthophylls from this source have absorption bands placed farther toward the red, having therefore a higher tinctorial power than the fish xanthophylls described by the present authors (also by Sumner and Fox, 1933, 1935*a*, 1935*b*, and Fox, 1936). Hence the determined values for concentrations of the latter xanthophyllic pigments are consistently somewhat lower than the true values, but these departures must be virtually uniform, since we were dealing with the same xanthophyll in all the fishes investigated to date.

† The rather well-developed young in this large, gravid female were discarded along with the viscera before analyses. It is very probable that this species, in common with most carotenoid-containing animals, transfers considerable quantities of the pigment from the body to eggs and young. This would account for the low figures in the particular specimen.

‡ I. e., 8 recta weighing 0.4 gram in the aggregate, excised from 8 fish, weighing 115.4 grams in the aggregate.

§ These recta were not weighed, but came from 8 fish weighing 120.0 grams.

ml.) to twice the weight (in grams) of the fish tissue extracted, giving uniform ratios of 2 ml. per gram throughout the series. In analyzing the recta, the pigment from the total (approximately 0.4 gram) of each lot was dissolved in 8 ml. ligroin, giving a ratio of 20 ml. per gram.

Table I presents a resumé of the experimental findings.

Observations made at the time that each lot of fish was sacrificed, revealed a progressive decrease in the pigmentation of rectal tissue, as shown by the difference between the xanthophyll content of the eight fishes' recta on the first day, and that of the eight sacrificed three days later. The carotenoid pigment found in the intestine proper was manifestly being constantly carried away in the blood, while that encountered in rectal tissue remained for longer intervals.

The apparent increase in body xanthophyll, noticed at about the ninth day, was without doubt due to a mere increase in relative quantity through loss of body weight following the refusal of food by the animals; the esterified xanthophyll of the skin tissues was evidently less readily catabolized than other body substances. Previous quantitative work by Sumner and Fox (1935*a*) showed that a different species of fish, *Fundulus parvipinnis*, which stores the same xanthophyll in esterified form in the skin and other body tissues, underwent no absolute loss of pigment when kept in the laboratory upon a carotenoid-free diet for three months.

Cymatogaster, when taken from their natural surroundings, gorged with food rich in carotenoids (including the xanthophyll which they themselves store), showed values of only 0.26–0.27 mg. xanthophyll per 100 grams of non-visceral body tissue, while the rectal tissues were usually brilliantly pigmented and the "stomach" colored to a less degree with carotenoids. Such animals kept for some days without food in laboratory aquaria maintained their relative quantity of body xanthophyll, while their rectal and intestinal carotenoid fell to nil. Should the rectal segment play the rôle of a storage organ for maintaining the supply of body xanthophyll, we might expect a prompt drop in the latter after the disappearance of the pigment from the former. Since the quantity of body xanthophyll neither builds up, beyond a rather low maximum, in nature when the supply in the food is plentiful, nor drops from this value in captivity when food is not consumed, it would appear certain that the uncombined xanthophyll temporarily stored in the rectal mucosa is not serving the purpose of resupplying the other tissues with pigment.

Since the rectal xanthophyll did, nevertheless, disappear rather soon, it seemed possible that it was being either (1) destroyed in situ, perhaps through the agency of an oxidase, (2) carried elsewhere by the

blood, and broken down into colorless compounds, or (3) secreted into the lumen of the rectum and subsequently eliminated.

To test the first possibility, several experiments were performed, using mixtures of aqueous extracts of isolated recta which had been preserved at freezing temperatures (although not sufficiently low to freeze the tissues), and both free and esterified xanthophyll in colloidal dispersion. A brief account of the experiments follows: 15 recta (nearly all of them distinctly red in color), stored for a few days at about —4° C. (not frozen), weighing about 0.2 gram, were triturated in a mortar with sand and phosphate buffer at pH 7.0. This pH was adopted because water extracts of *Cymatogaster* recta had shown pH values close to this value. Buffer solution was added to the extract to bring the volume to 10 ml. The solution was passed through a sintered-glass filter-funnel.

Free xanthophyll from *Cymatogaster* tissues was prepared by hydrolyzing the extracted esters. Whether the free or the esterified xanthophylls were used, the ligroin solvent was driven off in a current of gas, and the pigment residue was shaken vigorously with 10-ml. buffer (pH 7.0) solution to give a kind of emulsion. Equal volumes (1.5 ml.) of recta extract (previously boiled in the controls) were added to each 10-ml. system; 3 drops of toluene were introduced as bacteria-preservative, and the systems were allowed to stand at room temperatures in the dark.

At the end of 72 hours, the free xanthophyll was quantitatively recovered from both systems, ligroin solutions of each reduced to the same volume, and photometric measurements were taken in the usual way. The amounts of xanthophyll in both the experimental and the control were identical.

The corresponding xanthophyll ester systems were left for 117 hours, at the end of which time no diminution of pigment in the experimental as compared with the control had occurred; nor had the ester been hydrolyzed by the recta extract.

A similar experiment, using pH 7.0 buffer, and adding a few drops of olive oil to each vessel as an emulsifacient, keeping the systems at 37–38° C. for 21 hours, revealed no difference in quantity of pigment remaining in experiment and control.

Thus no oxidase for the destruction of xanthophyll was demonstrated in extracts of rectal tissue.

Other tissues of the body were not tested for xanthophyll oxidases in this way.

It should be said that red or orange-colored recta, stored at room temperature either (1) in diluted, toluene-preserved sea water, (2) un-

der ligroin or (3) directly under inert, colorless, heavy oil containing toluene as a preservative, were observed to lose their bright pigmentation in two or three days, and not by giving it up to the supernatant liquid. We are hardly prepared to state, however, that this was an oxidation hastened by an *enzyme,* since unesterified xanthophyll had been observed to fade about as readily *in vivo* (as demonstrated) or when extracted and stored in ligroin solutions.

The question next investigated was whether or not the pigment might be secreted by the rectal mucosa into the lumen of the organ, to be subsequently eliminated with the feces. A catch of *Cymatogaster* taken March 20 were brought to the laboratory and kept for about 24 hours, to permit them to expel all their feces. Animals sacrificed for examination at the end of this interval had red to orange-colored recta, but had voided all fecal masses. Thin pieces of wire whose ends were wound with cotton were carefully inserted into the rectum, gently revolved once or twice, and withdrawn. The cotton ends of these probes were still white save in one or two instances in which the tips were tinged with bits of yellowish mucus from the gut.

To apply a more rigid test, cotton plugs were inserted with forceps, one into the rectum of each of a dozen *Cymatogaster,* and the fish were kept in the laboratory aquaria overnight. The seven fish which were still living when examined in the morning had, with the exception of two which had expelled their plugs, retained the cotton in their recta for intervals ranging from 16 to 21 hours.

All five fatalities were females, gravid with young. The significant findings of the experiments are as follows: Notwithstanding the fact that all intestines were without food or feces, and that all save two rectal segments had faded from red or orange to pale yellow or white, all cotton plugs (including the two which had been expelled) remained colorless with the exception of two which were taken from dead animals, and showed yellow tinges. The dead group, unlike the living, all showed yellow mucus in the intestinal tract due to incipient sloughing following death. If in nature the rectal mucosa secreted its xanthophyll into the lumen, it would seem certain that the cotton plugs must have shown at least traces of yellow color. Since the plugs taken from the living fish (and from all save two of the dead) were entirely devoid of color, we conclude that the rectum does not eliminate xanthophyll by secreting it into the lumen.

Whatever may be the reason for the accumulation of free xanthophyll in the rectal mucosæ of perches which consume great quantities of shrimps, we are inclined to the belief that the tissue obtains its xantho-

phyll from the blood, and not by absorbing it directly from fecal matter in the lumen, for the following reasons:

(1) While the structure of the mucosa, both macroscopically and microscopically, is essentially the same throughout, the 5 mm. or so of the intestine just above the rectum is usually, though not always, colorless or nearly so. Were absorption continuous throughout the tract, there should not be, as there is, a distinct segment above the rectal sphincter usually devoid of color.

(2) The rectum may retain its bright orange color for several days after the fish have been brought into the laboratory and starved, while the rest of the intestine quickly loses it.

(3) In laboratory specimens of *Cymatogaster* or *Abeona* which have been recently fed carotenoid pigment in Garibaldi skin, shrimps, and *Emerita,* the upper intestine nearly as far back as the anal sphincter, characteristically assumes a more or less distinct (in some cases very pronounced) yellow or salmon color, while the rectum seldom does so, even in specimens in which the intestine, including the rectum, is full of food.

The frequent pigmentation of the rectum in nature, and its comparatively rare coloration in specimens fed in the aquaria are probably explicable as due to what we may call a " super-saturation " of the body of the fish in the former, as compared to its " under-saturation " in the latter situation, i.e., xanthophyll is, in the former case, supplied in such quantities and absorbed at such a rate as to exceed the rate of its destruction or elimination, hence it accumulates in blood probably up to a certain level of concentration and any surplus is then deposited in the rectal mucosa. In the laboratory, fish apparently did not usually consume sufficient xanthophyll-rich food, or at any rate did not absorb sufficient quantities of xanthophyll from it to build the concentration of the pigment in blood up to the point where it was deposited in the rectal mucosa pending destruction. In this connection, we are reminded of the yellowing of the shanks, ears, egg-yolks, and body fat of poultry fed upon a diet rich in xanthophyll (Palmer, 1922), the deposition of certain xanthophyllic carotenoids in the feathers of canary birds (Brockmann and Völker, 1934), the increase in the yellow color of cream and butter from cattle fed upon carotene-rich food, and finally the infrequently occurring condition in humans known clinically as xanthosis or carotenemia, in which carotenoids ingested in great quantities in the food are not disposed of rapidly enough by the body to preclude their deposition in the skin, which assumes a distinctly yellow color. The color disappears rather shortly after carotenoids are withdrawn or included more sparingly in the diet (Palmer).

The provisionally proposed paths of various carotenoids ingested in shrimps by *Cymatogaster* are given in summary form in Fig. 6.

Summary

1. A yellow, orange, or red pigmentation of the rectal segment of the intestine in certain surf perches is described and illustrated.

2. The carotenoid pigments of the shrimps consumed by the perch *Cymatogaster aggregatus,* the xanthophyll of the fishes' intestines and recta, and the xanthophyll ester, mainly of the skin, are described with respect to chemical and spectroscopic properties.

3. Carotene (apparently mainly the α-isomer), a red acidic carotenoid (not astacene), and a xanthophyll (or a mixture of two xanthophylls which were not separated) similar in chemical and spectral absorption properties to taraxanthin, are found in the shrimps (mainly *Hippolyte californiensis*). The acidic carotenoid and the xanthophyll(s) are present as epiphasic esters.

4. Both the red acid and xanthophyll are hydrolyzed and rendered hypophasic in the alimentary tract of *Cymatogaster,* but the xanthophyll(s) are apparently the only carotenoids absorbed by the intestine.

5. The carotenoid pigments of the rectal mucosa consist of the same xanthophylls as those in the shrimps, but unlike the latter, are stored in this tissue in unesterified condition. When the fish are not given food containing a supply of the carotenoid, the pigmentation of the rectal segment fades rather soon and may disappear completely in a few days.

6. The xanthophyll stored in the skin and perhaps in certain other tissues of the perch is re-esterified, and appears to be a single xanthophyll. It is small in quantity (0.26 to 0.27 mg. per 100 grams of wet, eviscerated fish tissue) and constant, whether the fish are gorged with shrimps or starved for some days or weeks in the laboratory.

7. Observations and experiments lead the authors to offer the following provisional conclusions regarding the metabolism of the xanthophylls of rectal tissue:

a. The carotenoid material is not absorbed directly from the lumen of the rectum by the rectal mucosa, but is taken up by the mucosa of the intestine proper, and carried by the blood to the rectal tissue; when present in the blood in concentrations which exceed some threshold value, the xanthophyll pigment accumulates in the rectal mucosa;

b. The rectum does not serve as a temporary storehouse to replenish the supply of skin xanthophyll, which is esterified and seems to be relatively stable and permanent;

c. It does not secrete the pigment into the rectal lumen to be eliminated with the feces;

d. It is apparently not an organ specially equipped with an oxidase enzyme for destroying the xanthophyll;

e. Its function may be that of a temporary repository of unesterified xanthophyll, which readily loses its pigment when a xanthophyll-rich diet is withheld.

8. Whether this excess xanthophyll is gradually excreted through channels other than the anus, or whether it is gradually destroyed (oxidized?) in the blood, the authors are unprepared to decide at the present time.

Acknowledgement.—Our thanks are due to Mr. P. S. Barnhart and Mr. C. I. Johnson of the Scripps Institution, and to various members of the Federal Works Progress Administration assigned to this project, who rendered valuable assistance in various routine aspects of this research.

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EFFECTS OF MECHANICAL DISTORTION ON THE STRUCTURE OF SALIVARY GLAND CHROMOSOMES

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It has been maintained by several authors [1] that the giant salivary gland chromosomes of Diptera are compound and that the striations or "threads" to be seen in the fixed chromosomes provide visible evidence of this compound structure. On such a view the "threads" are considered to represent chromonemata, and it is implied that these threads or chromonemata have a fixed size limit and that chromosome enlargement beyond this limit is regularly accompanied by division of the original threads. Without attempting to oppose this view as a theoretical conception, we have in earlier papers [2] presented evidence indicating, (1) that the visible striations or strands under consideration represent distortion effects rather than real threads or chromonemata, and (2) that if multiple chromonemata are present they are probably invisible and more numerous than the striations.

In those papers both chemical and mechanical distortion of the living materials have been considered briefly, and emphasis has been laid on the fact that whereas the striations or "threads" should be most conspicuous in chromosomes which have not been mechanically distorted, their prominence is actually proportional to the amount of twisting or stretching which the chromosomes have undergone. The present account is devoted to further consideration of the effects of mechanical distortion, especially those resulting from adhesions between different chromosome regions.

It is desired to emphasize the following five features here. (1) The protoplasm of these chromosomes is highly viscous and may be stretched into long, delicate strands or "threads." (2) These strands or "threads" of chromosome material may extend laterally or in other directions from a chromosome under suitable conditions of distortion, and may connect with and be indistinguishable from the longitudinal striations referred to above. (3) The chromatic materials appear to be more viscous and more tenacious than the achromatic. Apparently

[1] See Koltzoff, 1934; Bridges, 1934; Koller, 1935; Muller and Prokofieva, 1935; Bauer, 1935.

[2] Metz and Gay, 1934, 1935; Metz, 1935*a*, *b*; Doyle and Metz, 1935.

they are more resistant to distortion than the achromatic materials, but may be greatly attenuated if subjected to sufficient tension. (4) By means of mechanical distortion local rearrangements of materials within a chromosome may be produced which result in striations having the same characteristics as the longitudinal striations, but which are evidently artifacts. (5) The number of strands or striations may vary widely in adjacent regions of a chromosome, and vary in such a way as to indicate that they are artifacts rather than a reflection of preexistent structures in the living chromosome.

These features will be illustrated by a few examples from a large number available. All are from fully developed glands of *Sciara ocellaris* Comst. The accompanying drawings were all made from aceto-carmine preparations, with the exception of Fig. 2*a*, which is from an osmic-aceto-carmine slide. All the figures except the sketch shown in Fig. 1*b* were made by drawing with India ink directly on photographs, and then bleaching the prints. They are necessarily schematized somewhat, since the photographic details cannot be reproduced in plain black and white; but the features under consideration show even more clearly in the photographs than in the drawings. Only features of present interest have been included. It is planned to publish the photographs in a later paper. The drawings were made at a magnification of 3,000 ×, and are reduced to approximately 2,000 × in publication. Acknowledgment is made to Mrs. Elizabeth Gay Lawrence and Mrs. Arlene DeLamater, respectively, for assistance in making the slides and the drawings.

The first-mentioned feature is illustrated in Fig. 1. One localized region of the chromosome shown in this figure was so greatly stretched, in making the smear, that the material became attenuated into a single delicate strand. The delicacy and continuity of this strand show how cohesive the chromosomal constituents are and how greatly the material may be stretched. This single strand exhibits thickenings or "granules" which represent the remains of thickenings or "granules" in the "discs" before the stretching occurred. (It is to be remembered that the material was coagulated by fixation before being subjected to mechanical distortion.)

The upper part of this figure also illustrates the fourth feature mentioned—a rearrangement of materials within the chromosome, and the production of striations by distortion. This aspect will be made more evident by examination of the outline sketch (Fig. 1*b*) showing how the chromosome has been distorted. An adhesion has been formed between one end of the chromosome, at *a*, and the side of the chromosome, at *b*. The distorted region (*c*), therefore, is being held in posi-

tion to some extent by this adhesion. The transverse bands or discs are in normal position a short distance from this distorted region, but show some distortion in the region marked *d*, and progressively greater distortion in regions *e* and *f*, the latter being the region shown in the larger drawing. In this tapering region, from which the single strand extends, the materials of the chromatic discs, together with the intervening achromatic materials, have been rearranged by the tension so as to produce granular striations (separated by rows of alveoli) focusing on the point of tension. It seems clear that these striations do not represent preëxistent structures, either longitudinal or transverse. The evidence also supports other lines of evidence in indicating that the inter-alveolar, chromatic material is more viscous than the alveolar, achromatic material.

An illustration of the effects of moderate stretching is shown in Fig. 2 and an intermediate condition between this and the one just described is shown in Fig. 3. The region shown in Fig. 2*b* has been stretched only slightly—just enough to elongate the droplets or vacuoles so that they appear hexagonal in optical section. The "honeycomb" structure is conspicuous in cases like this when enough chromatic material is distributed around the vacuoles to reveal them distinctly. With other types of treatment (see Metz, 1935*b*) the chromatic material may be more definitely restricted to the transverse discs, and the outlines of the vacuoles correspondingly less distinct. The region shown in Fig. 2*a* has been stretched a little more. Distortion here has been sufficient to obliterate the more delicate transverse bands, but not enough to obscure the heavier ones or to destroy the internal organization. It seems clear from our observations that the "longitudinal striations" observed in more severely stretched chromosomes are derived from the

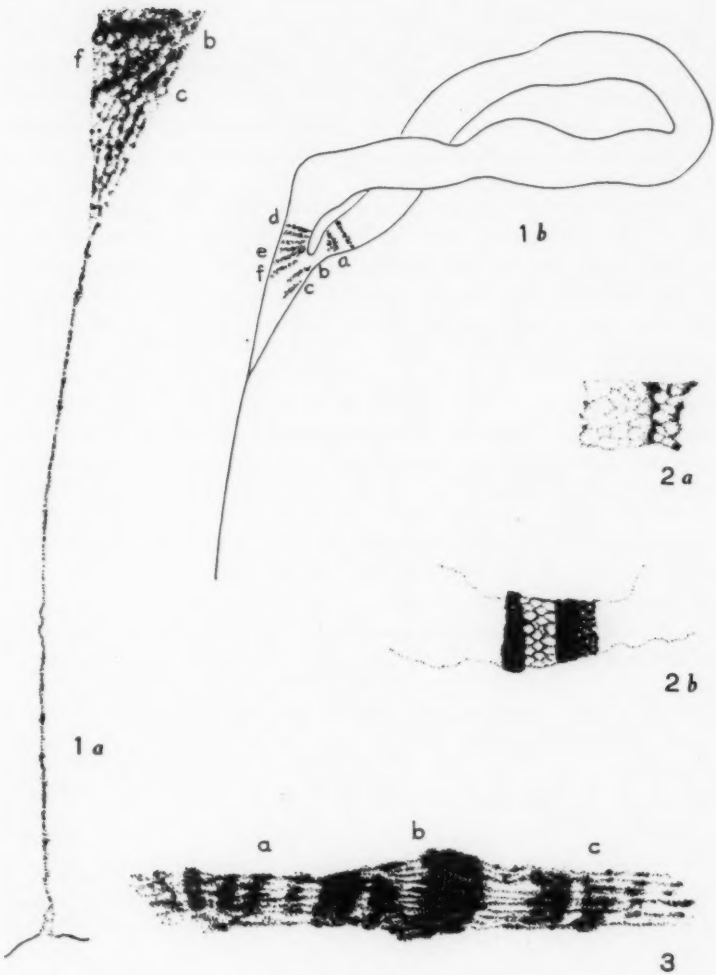
EXPLANATION OF FIGURES

FIGS. 1–3. Illustrating the effects of different amounts of distortion on salivary gland chromosomes.

FIG. 1. The chromosome shown here was pulled almost in two, the two parts remaining connected by the delicate strand shown in the figure. The lower end of the strand is apparently connected with the rest of the chromosome, but the latter is entangled with several others and is not represented in the figure. The wavy outline of the strand in the lower part is not due to lack of tension, but to achromatic droplets or vacuoles, around which it extends. See text for further details. 1*a*, detailed drawing. 1*b*, outline sketch.

FIG. 2. Two regions, slightly stretched, showing the distorted alveolar structure which appears in the form of a network at any one optical level, or a honeycomb when considered in three dimensions. *a* from an osmic-aceto-carmine preparation; *b* from an aceto-carmine preparation.

FIG. 3. Severely stretched portion of a chromosome in which the more delicate structures have been entirely disrupted and the coarser ones disarranged. The finer bands have disappeared and the chromatic material has been pulled out into striations or strands, as described in the text.



FIGS. 1–3

lines observed in moderately distorted cases like those shown in this figure. These lines, however, form a network at any one focal level. No discrete, continuous lines of thread-like nature are visible. The structure appears definitely to be honeycomb-like when considered in three dimensions. Comparative study of material fixed in different ways indicates that the compartments of the honeycomb represent the achromatic droplets or vacuoles seen in less distorted material between the cross bands or extending through the cross bands if the latter are represented by granules.

The short lines extending longitudinally between two successive cross bands (discs or rows of granules) apparently represent chromatic material lying between the droplets or vacuoles which occupy this space. Such chromatic material presumably flowed out from the chromatic discs or else became separated from the achromatic material in the process of coagulation. In any event it does not seem to represent threads, but rather to represent the walls of the droplets or vacuoles. These features will be considered more in detail in another paper.

With further stretching the structure just considered becomes more and more distorted, as indicated in Fig. 3. The achromatic material appears to be the first to become disrupted, perhaps because it is in the form of separate droplets. The more delicate parts of the network or honeycomb become broken up, and most of the chromatic material at least becomes drawn into longitudinal striations, with traces of the original type of structure remaining only here and there.

It is to be noted that the number of strands is not uniform in the chromosome shown in Fig. 3. The drawing represents only one focal level, but the relative numbers of the striations are indicated fairly accurately. Careful study of all focal levels in the different regions indicates that in some regions, such as those marked *a* and *c*, the total number of striations ranges from 7 to 10. In contrast to this condition, the region at *b*, lying between *a* and *c*, shows a much larger number of striations. There appear to be more than 20 at this location, including all focal levels.

If the striations are assumed to represent preëxistent threads, this discrepancy might be interpreted as being due to breakage of some of the threads in such regions as *a* and *c*, or to previous additional division of the threads (chromonemata) in such regions as *b*, giving regions of the latter type a larger number. We have been unable, however, to find support for such an interpretation in studying the preparations themselves.

According to our observations, a thick region such as that shown at *b*, in a stretched chromosome, represents either a series of heavy chro-

matic discs which resist stretching, or else one of the "expanded" and disorganized regions often found in aceto-carmine preparations (see, e.g., Metz, 1935*b*, Fig. 5*A, B, C*). In the former case the resistance to stretching results in less distortion and in a retention of more of the "network" than in surrounding regions. Hence more striations are seen in such regions. In the other type, representing an "expanded" region, the original organization of the chromosome (in such a region) has been broken up by fixation and then the resulting structure has been further distorted by stretching. The fixation process results in the formation of an expanded region in which granules and vacuoles are intermingled, apparently at random. Not all preparations show this effect. At any one transverse level in such a region many vacuoles and granules are found. With moderate stretching a correspondingly large number of short striations appear. These apparently represent the boundaries of the vacuoles, and the large number is interpreted, not as an indication of the presence of many threads in the region, but as a reflection of the number of vacuoles, as just intimated.

When not subjected to mechanical distortion the expanded regions often show a more or less uniformly granular structure, without longitudinal striations. It is difficult to see how a structure of this type could thus appear if the chromosome originally contained numerous discrete longitudinal threads (chromonemata) unless these threads had become disorganized. If the latter assumption is made, however, it would carry with it the assumption that no threads would appear in such regions if they were stretched after fixation. But stretching does result in the apppearance of striations here, indistinguishable from those of other regions; and, as in the case of other regions, the direction of the striations coincides with the direction of stress.

The characteristics of these expanded regions are those to be expected on the present view, which considers the *fixed* chromosome as made up of continuous chromatic material, confined mainly to the discs, and discontinuous achromatic material in the form of droplets or vacuoles lying mainly between the discs. Expansion of the chromosome would be expected to give rise to a granular, vacuolated structure, without striations, but one in which striations could be produced by distortion just as in other regions. Furthermore, since the striations should, on this view, be numerically proportional to the number of droplets or vacuoles in a transverse section of the chromosome, there should be more of them in expanded than non-expanded regions. This agrees with the findings just considered and with those illustrated in Fig. 3.

Several cases have been examined in which an expanded region in one chromosome has adhered to the side of another chromosome and the two have then been pulled apart when the cell was crushed. In such cases that part of the expanded portion which is subjected to tension shows a realignment of materials to form striations, focusing at the point of adhesion. In some of these cases similar rearrangement of materials and focusing of the striations has been effected in the other

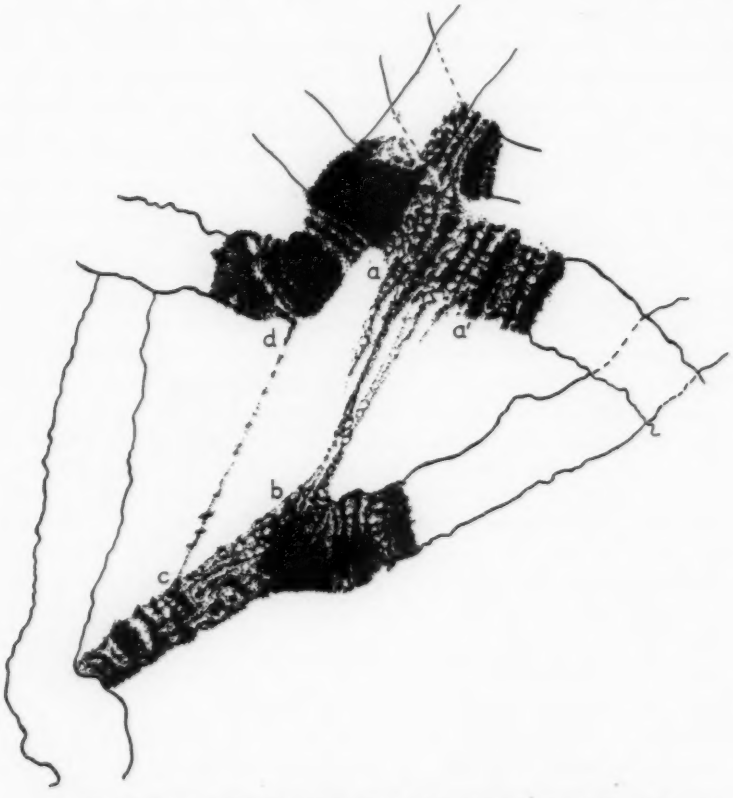


FIG. 4. Illustrating the distortion effect of lateral adhesion followed by mechanical separation of the two chromosomes. Chromatic materials from the bands between *a* and *a'* in one chromosome have been drawn out at right angles to the chromosome in the form of strands, connecting with similar strands from the other chromosome, at *b*. Such distortion should reveal the presence of longitudinal "threads" within the chromosome if detectable chromonemata are present; but none appear.

chromosome where no previous expansion had occurred. Between the two chromosomes extend strands of material similar to the striations. In these cases, as in the others just cited, it seems clear from the location and direction of the striations that they cannot represent pre-existent threads or chromonemata.

Fig. 4 provides further illustration of these features and also of the fact that the chromatic materials may be drawn out into threads extending at right angles from the chromosome. The two chromosomes represented at *a* and *b* in this figure had adhered laterally and were then pulled apart immediately after fixation. It is evident that materials from the chromatic discs between *a* and *a'* have been drawn out laterally in the form of strands and that these strands could not have been pre-

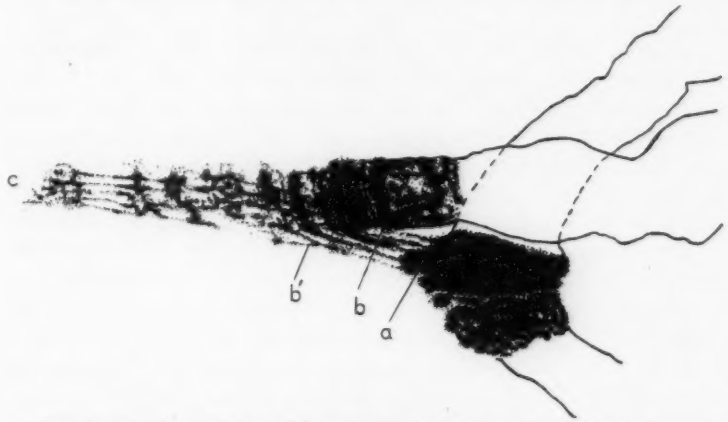


FIG. 5. Another example of lateral adhesion followed by mechanical separation of the two chromosomes. See text for description.

existent as longitudinal threads in the original chromosome. It seems particularly significant also that there is no indication in such cases of bent longitudinal striations in the chromosomes, such as would be expected if the chromosomes were full of longitudinal threads before distortion.

The resemblance of these lateral strands to longitudinal strands or striations within the stretched chromosome may be seen by comparison with the structures shown in the stretched region between *b* and *c*, in the lower chromosome. Another strand connecting two separate chromosomes is evident between *c* and *d*.

A particularly interesting example of lateral adhesion followed by stretching is represented in Fig. 5. Before the cell was crushed an

adhesion had evidently formed between the thickened part of the lower chromosome at *a* and the region in the upper chromosome between the points marked *b* and *b'*. Then, in crushing the cell, the two chromosomes have been pulled somewhat apart in the region of adhesion due to a movement of the upper chromosome toward the left. Materials from the chromatic segments between *b* and *b'*, and perhaps to some extent also from segments in *a*, have been drawn out in the form of strands or threads. Chromosome *b* has been stretched toward the left, producing conspicuous longitudinal striations or strands which are indistinguishable in visible characteristics from the lateral strands connecting the two chromosomes. It seems especially significant that the lateral striations, which are obviously the products of distortion, not only resemble, but connect with, the longitudinal strands within the chromosome. Another feature of special interest to be noted here is the fact that the strands or striations do not extend strictly longitudinally in the upper chromosome but follow a somewhat diagonal path from *c* toward *a*. In other words, the lateral strands between the chromosomes, and those within the upper chromosome itself, coincide in such a way as to indicate clearly that they are both due to the tension between regions *a* and *c*. It is difficult to avoid the conclusion that these strands are all of the same type and are all artifacts produced by distortion.

Discussion

The relation of the accompanying evidence to current hypotheses of chromosome structure will be considered in another paper. Brief mention of a few pertinent points, however, may be made at this time. It seems significant, not only that strands of chromosomal material which are evidently artifacts may resemble and connect with the "striations" within the stretched chromosome, but also that when strands are pulled out at right angles, as shown in Fig. 4, the distortion reveals no evidence of any longitudinal "threads" within the chromosome itself. If definite threads were present such distortion should bring them into view in the form of parallel *V*'s. Each longitudinal thread near the distorted edge should be pulled out somewhat, laterally, making a flattened *V*. We have numerous cases of the type represented in Fig. 4, in which materials from the discs have been pulled out at right angles, and none of them reveals any evidence of longitudinal thread-like structures inside the chromosome.

Such evidence tends to indicate not only that the "striations" are not chromonemata, but that no multiple chromonemata are present in the chromosome, unless such chromonemata are extremely small and delicate and are invisible in preparations thus far studied.

Our previous evidence has weighed strongly against the view that conspicuous chromonemata are present extending spirally around the periphery of the chromosome, as postulated by Koltzoff, and also against the view that conspicuous, essentially straight chromonemata fill the entire chromosome. It seemed possible, however, that a large number of chromonemata might be present if each were assumed to be independently and closely coiled. Such a structure might possibly give the appearance of a network or honeycomb such as we have seen in the fixed chromosomes; and when stretched it might give a striated appearance due to straightening out of the fine coils. If such threads were present, however, they should be revealed in cases such as those just considered; yet the preparations show no indication of anything of the kind.

Another feature, bearing indirectly on the above considerations, may also be mentioned at this point. In earlier papers (Metz, 1935*a*, *b*) it has been suggested that in the living chromosome, before shrinkage, the chromatic materials may be present in the form of continuous, relatively smooth discs, separated by intervening discs of achromatic material. Further comparative study of the effects of different fixatives, however (considered more in detail elsewhere), suggests the *possibility* that in the normal living chromosome no such sharp distinction exists between these two types of materials, and that the distinction in the shrunken chromosome is due to separation of components as a result of precipitation and coagulation. Such an interpretation, if substantiated, would indicate that the chromosome is composed of a series of different materials, representing the genes, but that they are not so sharply differentiated and isolated from one another as formerly supposed. Numerous serious difficulties stand in the way of such an interpretation, of course, and it is not desired to stress it on the basis of the present meager evidence. It seems worth while, however, to call attention to it and to its possible bearing on phenomena of crossing over and of "position effects" of different genes. If no sharp boundary lines existed between successive genes, crossing over would presumably be effected by the genes themselves, rather than by "inert" material between them; and the interaction of genes in close proximity to one another ("position effect") would be subject to a different interpretation than that based on the current conception of distinctly separate units.

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THE RÔLE OF BLOOD CELLS IN EXCRETION IN ASCIDIANS

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How do ascidians dispose of the waste products resulting from purine metabolism? They have no tubular kidney comparable to the excretory organs present in many animals. The neural gland, the pyloric gland, and the terminal portions of the sperm ducts have been suspected of having an excretory function; but such suspicions rest on slight evidence, and are not generally credited. Some species have renal vesicles. When present, these are generally recognized as subserving a kidney function; but they are not present in all or even in most species of ascidians.

The renal vesicle in the Molgulidæ is a single large, closed, bean-shaped sac in which waste matter is gradually deposited and stored in the form of concretions throughout the life of the animal. In most members of the family Ascidiidæ, and probably in some other families, there is, instead of one such kidney, a multitude of minute vesicles containing concretions. Structurally these concretions consist of concentric lamellæ and they may be simple or compound. Chemically they have been shown by means of the murexide test to contain uric acid (Kupffer, 1872, 1874; Lacaze-Duthiers, 1874). According to Dahlgrün (1901), who has studied the histology of these excretory organs, the vesicles have an epithelial wall one cell thick. A characteristic of the epithelial cells is the presence of an extensive system of fluid-filled vacuoles. Azéma (1926, 1928) has studied the structure and activity of isolated living vesicles of *Ascidiella aspersa* Mül. He finds that the cells forming the walls of the vesicles in this species always have a large vacuole near the nucleus and frequently young vacuoles in process of formation. In living material one may observe small concretions form upon the inner wall of the large vacuole, detach themselves, and float freely in it. By rupture of the main vacuole of a cell its concretions are expelled into the cavity of the multicellular renal vesicle. A small young vacuole then begins to increase and becomes substituted for the vacuole which ruptured.

In those ascidians that have no renal vesicle, and these are probably

the greater number, the renal function appears to be taken care of by cells of the connective tissue and blood. Herdman (1888) mentioned the occurrence of excretory cells in *Botryllus,* and Roule (1884) reported cells of *Ciona intestinalis* that produced the characteristic reactions of uric acid and uric acid salts. They had thin walls and contained very small granules. Groups of these cells located in the connective tissue of the wall of the vas deferens he considered to be the kidney. Dahlgrün (1901) described the excretory organ of the Botryllidæ as consisting of a rather large number of isolated cells having an oval nucleus and containing bright brownish granules. He found these cells in the visceral region, especially in the space between the œsophagus and the stomach on one side and the rectum on the other side, usually immediately adjacent to the gut wall in the meshwork of stellate mesenchyme cells. In serial sections of *Ciona intestinalis* he found these cells with concretions in the visceral region only in the immediate neighborhood of the gut. They were present in small or large numbers depending upon the age of the animal.

More recently the findings of Azéma and my own observations show that these cells with granules of an excretory nature are blood cells and that they may circulate freely in the blood stream or be localized in the connective tissue.

In the course of comparative studies of ascidian blood I have observed blood cells with one or several vacuoles practically filling the cell body. Suspended in the fluid of the vacuoles Brownian granules are usually but not always present. In some species these granules are always colorless, as in *Perophora viridis;* in others they are colored. Some species may have cells with colorless granules and also cells of the same structure but of two or more color varieties, as is the case in *Clavelina picta.* Judged by the contents of the vacuoles, cells of this structure may be of at least two functional varieties: nutritive cells and excretory cells. In earlier papers (George, 1926, 1930*a*, 1930*b*) I described compartment and signet ring cells having granules of a fatty nature in some species. Cells with essentially similar structure may contain granules that are not of a fatty nature but apparently of purine composition.

Azéma (1928, 1929*a, b, c, d*), on the basis of various microchemical reactions, concludes that granules in vacuolated cells of the genus *Microcosmus* of the family Pyuridæ and in some species of the family Synoicidæ are composed of xanthine. The white pigment in certain cells of the Botryllidæ he calls a purine without specifying which one. In *Ascidia pellucida* he finds vesicular cells with concretions which present the distinctive characteristics of guanine. It is of some sig-

nificance to note here that Sulima (1914) made a quantitative analysis of the purine content of the body tissues of *Cynthia microcosmus* (*Microcosmus vulgaris*). From his analyses he found a mean value of about 0.2 per cent uric acid and 0.3 per cent other purine bases (calculated as xanthine).

Within the living excretory cells the granules have certain characteristic optical qualities. By oblique or reflected light they shine in a dark background. This is a characteristic found in purine containing cells of other animals. Millot (1923), who made a detailed study of the guanophores of the lower vertebrates, found that crystals of guanine in the cells took on a brilliant aspect against a dark background. The reflecting granules in ascidian blood cells are one color by transmitted light and another color by reflected light. Such two-color granules, which may vary in size from the limits of visibility with the highest powers of the microscope up to concretions several microns in diameter,

FIG. 1. Excretory cells of ascidian blood. *a*, *b*, and *c* are vesicular cells with reflecting concretions from the blood of *Polyandrocarpa tincta; b* is a young stage. *d* is a vesicular cell with reflecting granules from the blood of *Pyura vittata.*

I have found in *Clavelina picta, C. oblonga, Distaplia bermudensis* of the family Polycitoridæ; in *Symplegma viride, Polycarpa obtecta,* and *Polyandrocarpa tincta* of the family Styelidæ; in *Pyura vittata* of the family Pyuridæ; in *Botryllus schlosseri* and *Botrylloides niger* of the family Botryllidæ. In *Ecteinascidia turbinata* Herdman and in *E. conklini minuta* Berrill of the family Ascidiidæ there are similar cells, but I could not see the intravacuolar granules in the living cells because of the presence of a film at the surface of the vacuoles. This film, like the granules in other species, was brown by transmitted light and white or yellowish white by reflected light. All of the above listed species have pigmented cells that are brown by transmitted light and white by reflected light. Some of them have in addition other two-color pigment cells. In *Distaplia bermudensis,* in addition to the typical brown and white cells, cells are present with granules that are purple by transmitted light and black by reflected light and also cells that are

yellowish brown by transmitted light and black by reflected light. Perhaps some impurity in the purine granules accounts for these variations.

Though commonly present in the form of granules (Fig. 1*d*), the reflecting pigments in ascidian blood cells may exist as simple or compound concretions (Figs. 1 *a, b, c*), as in *Polyandrocarpa tincta* and *Bolteniopsis prenanti.* The resemblance of these concretions to renal calculi is very striking. They are, in fact, microscopic intracellular calculi. They begin as tiny granules formed within cell vacuoles and grow by the addition of substance at the surface of these granules. Various stages in their development may be observed in living cells.

The vacuolated cells with purine granules and concretions seem to originate through differentiation of lymphoid cells and probably of fixed mesenchyme cells. Millot (1923) derives the guanophores of the lower vertebrates likewise from connective tissue cells and leucocytes of undetermined origin.

These excretory cells are present in the blood stream, but they need not remain in circulation. In *Ecteinascidia conklini minuta* I have observed them circulating in the blood spaces and also collected in clumps at the tips and near the bases of the siphon lobes, and collections of these cells cause flecks of white near the anterior end of *Clavelina oblonga* Herdman and here and there on colonies of *Botrylloides niger.*

Different species within the same family may have in one case multicellular renal vesicles, and in another, vesicular renal cells in the blood and connective tissue. Thus *Ascidia hygomiana* and *A. nigra,* according to my observations, and *A. mentula* and other species reported by Dahlgrün (1901) and other authors, have numerous multicellular renal vesicles with concretions, while *A. pellucida* is devoid of renal vesicles but has in the visceral region and in all the body wall pigment spots composed of vesicular cells containing concretions, which in this form present the distinctive characteristics of guanine (Azéma, 1929*d*). Also in the Pyuridæ Dahlgrün records renal vesicles for *Cynthia dura* and *Microcosmus serotus,* while Azéma (1929*a*) reports a number of species in which there are no renal vesicles but renal cells with purine concretions. I have been unable to find renal vesicles upon examination of serial sections of *Pyura vittata;* but renal cells are present. It seems not improbable, however, that renal vesicles and renal cells may be found in the same individuals of some species.

It seems evident from the foregoing facts that the ascidians make use of their blood cells in freeing the tissue fluids of the wastes of purine metabolism. Excretion granules have been found in free cells of other invertebrates, and even in the higher vertebrates it appears that blood cells participate in the excretory function. Bornstein and Griesbach

(1919) and Theis and Benedict (1921), upon examining many samples of blood, found that in some cases the quantity of uric acid was greater in the plasma and in others greater in the cells, and Benedict (1915) found that in ox blood uric acid is largely confined to the erythrocytes, though in chicken blood it is almost wholly in the plasma. The mode of its occurrence has been a matter of interest to biological chemists. Rose (1923), in his review of purine metabolism, calls attention to the fact that Bechold and Ziegler (1914), in order to explain the presence of more uric acid in the blood than the solubility of the lactim form accounts for, assumed, but did not demonstrate the presence of urates in colloidal condition. The demonstration of oxypurines in colloidal suspension in the vacuoles of ascidian blood cells gives some factual justification for Bechold and Ziegler's assumption.

There is another point in which the conditions in the ascidians may have some significance with regard to our conceptions of the nature of the excretory process in vertebrates. Partly because of its thinness and the absence of cytological evidence of secretory activity, there has been a tendency to look upon the epithelium of the renal corpuscle as being merely a filtration membrane. This assumption may be entirely correct; but the excretory activity of ascidian blood cells shows us that we are not justified in denying a secretory function to the glomerular epithelium merely because of its thinness. For in these cells we have a protoplasmic membrane so thin as to be merely a thin line under the highest magnification, and yet it appears to function as a glandular membrane, taking purine substances out of the blood plasma and storing them in the cavities of these unicellular bladders.

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